Advances in Automated Sample Preparation for Gas Chromatography: Solid-Phase Microextraction, Headspace-Analysis, Solid-Phase Extraction

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Summary

Despite the widespread use of more and more effective detection technologies such as tandem-mass spectrometry, it is becoming increasingly clear that proper sample preparation is still the key to a successful analysis. The most important demands are thereby complete automation, sensitivity, reliability, economic efficiency and also implementation of “green” technologies that conserve our environment, e.g., by avoiding the use of larger volumes of organic solvents. Modern microextraction techniques can fulfill these demands and are already capable of replacing many traditional, manual sample preparation approaches. This thesis presents advances in the field of microextraction technologies. The primary scope is thereby the development and evaluation of a novel solid-phase microextraction device (SPME). PAL (prep and load) SPME Arrow retains all advantages of the classical SPME fiber and augments this proven concept with improved mechanical reliability and sorption phase volume. Initial evaluation of the method shows significantly increased extraction yields and a potential to further advance the field of SPME. Polycyclic aromatic hydrocarbons (PAHs) could be analyzed from the freely dissolved fraction in water with detection limits that are at least one order of magnitude better as in case of the classical SPME fibers. In the field of headspace analysis (HS), a systematic comparison between available techniques has been carried out using 41 volatile analytes. The results clearly show how enrichment techniques such as in-tube extraction (ITEX), SPME or PAL SPME Arrow deliver better sensitivity than static headspace. The instrumental mode of action is thereby of larger influence to method sensitivity and reproducibility than its type of automation. This is demonstrated by correlating the results of a loop and trap sampler to those generated with the syringe-based static headspace option and various enrichment methods. Sorption phase material selection had the smallest influence in this context and the beneficial potential of special sorption phases seems to be limited to the analysis of compounds capable of specific molecular interactions. As for the classical solid phase extraction (SPE) the novel ITSP (instrument-top sample preparation) option in terms of miniaturized cartridges (MSPE) has been evaluated. By using these, the complete sample preparation in terms of extraction, elution and further refinement such as evaporation and derivatization can be carried out by PAL-type samplers. First results indicate that this option has a lot of potential but still requires further improvement, e.g., in terms of time efficiency.

Zusammenfassung

Trotz der Nutzung immer leistungsfähigerer Detektionstechniken wie der Tandem-Massenspektrometrie, wird stetig klarer, dass gute Probenvorbereitung nach wie vor der Schlüssel zu einer erfolgreichen Analytik ist. Die wichtigsten Anforderungen sind dabei vollständige Automatisierbarkeit, Empfindlichkeit, Zuverlässigkeit, ökonomische Effizienz und der Einsatz „grüner“ Technologien welche die Umwelt schonen, z.B. durch den Verzicht auf größere Mengen organischer Lösungsmittel. Moderne Mikroextraktionstechniken können diese Anforderungen erfüllen und dabei bereits zahlreiche, klassische, manuelle Probenvorbereitungstechniken ersetzen. Diese Arbeit präsentiert neue Entwicklungen im Feld der Mikroextraktionstechniken. Der primäre Fokus lag dabei auf der Entwicklung und Evaluation einer neuartigen Festphasen-Mikroextraktionstechnik (SPME). PAL (prep and load) SPME Arrow behält die Vorteile der klassischen SPME-Faser bei und wertet diese um bessere mechanische Zuverlässigkeit und größere Sorptionsphasenvolumina auf. Erste Evaluationen dieser neuen Technik zeigen erhebliche Zugewinne an Extraktionsausbeute, sowie Potential das generelle Feld der SPME weiter zu entwickeln. Dabei konnten polyzyklische, aromatische Kohlenwasserstoffe (PAKs) aus der frei im Wasser gelösten Fraktion bestimmt werden, wobei Nachweisgrenzen erreicht wurden die mindestens eine Größenordnung besser waren als im Falle der klassischen SPME-Faser. Im Umfeld der Headspace-Analytik (HS) wurde anhand von 41 volatilen Analyten ein systematischer Vergleich von sechs Probenvorbereitungsoptionen durchgeführt. Dabei wurde klar, dass Anreicherungsverfahren wie In-Tube Extraction (ITEX), SPME und PAL SPME Arrow im Hinblick auf die Empfindlichkeit den statischen Headspaceverfahren überlegen sind. Das Operationsprinzip ist dabei klar wichtiger als die Art seiner Automatisierung, was durch Gegenüberstellung eines Loop- und Trap-Samplers mit einer spritzenbasierten, statischen Headspace Option sowie verschiedenen Anreicherungsoptionen gezeigt werden konnte. Die Auswahl unterschiedlicher Materialien als Sorptionsphase hatte dabei klar den geringsten Einfluss und scheint eher für Analyten wichtig zu sein, welche zu spezifischen, molekularen Interaktionen fähig sind. Für die klassische Festphasenextraktion (SPE) wurde die neuartige ITSP (instrument-top sample preparation) Option Evaluiert. Mit diesen miniaturisierten Kartuschen lässt sich der komplette SPE-Ablauf samt einer Einengung und Derivatisierung komplett auf einem PAL-Sampler abbilden. Erste Resultate zeigen neben dem großen Potential dieser Option, noch bestehenden Bedarf an Optimierung z.B. bzgl. der zeitlichen Effizienz.

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1 Introduction and scope

1.1 Modern demands in instrumental analytics

Despite the fact that instruments for the chromatographic separation and detection of compounds show constant improvement with each new cycle of innovation, the preparation of samples prior to their injection into those instruments has often been neglected in the past[1,2]. While considerable options have already been available in the field of microextraction techniques[3], especially routine laboratories often continue the use of classical sample preparation methods such as liquid-liquid extraction (LLE). Typical reasons for this are that the staff is already well trained in such methods, which are in addition also specified in various quality-control procedures. Furthermore, doubts may arise concerning the comparability of results when such methods are replaced by modern, automated alternatives.

In many cases, this delayed adoption of improved methodological alternatives may have caused superfluous use of large volumes of organic solvents and working time. This situation can, however, be expected to change in the near future, since especially the latter becomes an increasingly important cost factor in laboratories worldwide. Organic solvents on the other hand are often expensive, toxic, harmful to the environment and ozone layer and create additional work and cost due to proper storage, handling, waste collection and subsequent disposal[4]. All these aspects, combined with the general rise in environmental awareness create further pressure on laboratories, to minimize their use of organic solvents.

Sample preparation has always been the potentially most time-consuming and error-prone part of the analytical process. While sample injection, the measurement sequence and subsequent data processing and output of results are typically well-automated and mostly reliable today, the principle “garbage-in = garbage-out” describes the impossibility of generating any useful results if the previous sample preparation was not carried out properly[4]. According to this principle, mistakes during initial sampling and sample preparation can hardly be corrected for afterwards, even with the most sophisticated measuring equipment.

Modern solventless sample preparation techniques such as microextraction options are capable of offering solutions to the demands of repeatability, automatability, avoidance of solvents and manual steps. More reproducible and comparable results can be generated in shorter time with less workforce, monetary effort and environmental impact[4].

Such techniques typically achieve these benefits by the use of an intermediate transport medium, into or onto which the desired analytes are transferred (sometimes selectively) in order to abstract them from their matrix. This transport medium is for example air in terms of headspace gas above the sample liquid in case of the headspace analysis technique. For solid-phase microextraction (SPME), a polymeric sorption phase is used as transport medium, which is immobilized on a specially designed fiber. It is first exposed to sample liquid or headspace for enriching analytes and afterwards transferred into the heated injector for desorption of the analytes. There are many combinations and variations of these techniques, for example with the polymeric phase being applied as a coating to the internal surface of a syringe or around stir bars. But the general mode of action always remains the same with the goals of matrix separation and often also enrichment of analytes in mind.

The most important demands for microextraction techniques are reliability, user-friendliness, a large sample capacity, sensitivity, precision and minimized application of laborious, manual procedures and organic solvents. This is sometimes in contrast to the increasing complexity of analytical demands[5] and requires advances in laboratory automation. The latter requirement has triggered the development of a novel generation of autosamplers, which now have the ability to automatically switch between different tools that enable various interactions with the samples.

Besides the automation of sample preparation procedures such as derivatization, internal standard addition or LLE (Figure 1), which were mostly carried out manually so far, these new capabilities also enable flexible implementation of microextraction techniques[6] and combinations of all these procedures during individual sample preparations. As for the microextraction methods, updated variants of well-established techniques such as SPME[7], in-tube extraction (ITEX)[8] and solid-phase extraction (SPE) are being developed for this new generation of autosamplers.

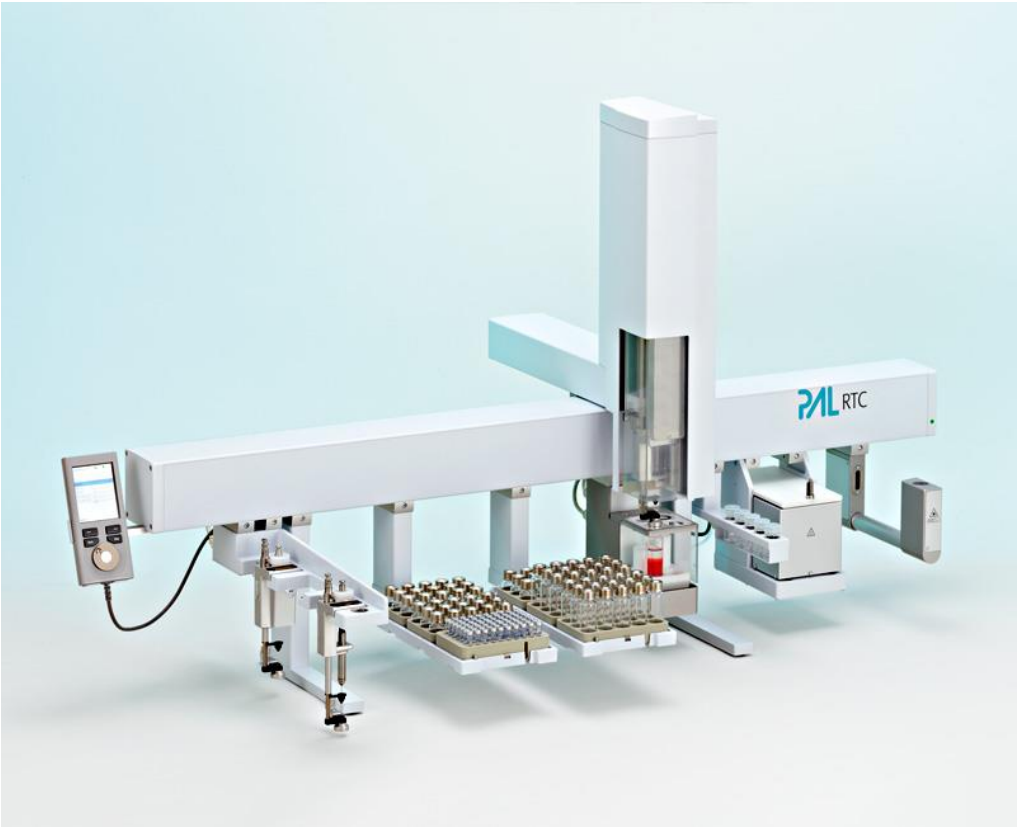

Figure 1: CTC PAL RTC (robotic tool change) equipped with automatically selectable tools for headspace analysis, solid-phase microextraction and a liquid syringe that is currently performing a liquid-liquid extraction in an attached vortex mixer module. Further attached modules (from left to right) are: Tool park station, Sample trays, a syringe wash station, an agitator and a reading module for barcodes on sample vials that may, e.g., contain individual method parameters. Picture kindly provided by CTC Analytics AG

1.2 Background of microextraction methods

Solventless sample preparation is based on the replacement of liquid organic solvents with an intermediate, additional phase for abstraction and temporal handling of analytes apart from their original matrix. This role is fulfilled either by gases in case of headspace extraction or by solid sorbents in case of microextraction techniques. Latter typically polymeric phases altogether share the property of a significantly smaller volume compared to the original sample volume - hence the name micro extraction technique[2]. The sampling process itself is thereby based on a (not necessarily complete) equilibration of analyte concentrations between their original solution or its headspace and the extraction phase.

The occurring partitioning is thereby based on absorptive processes (in case of polymers such as polydimethylsiloxane (PDMS)), adsorptive processes (in case of solids such as Carboxen) or a mixture of both (in practice, e.g., Carboxen sorption phase consist of solid particles embedded into PDMS).

One of the most apparent advantages of solventless concepts over, e.g., classical LLE is that this intermediate extraction phase is either entirely cost-free and renewed with each sample in case of headspace extraction, or at least regenerative for a couple of hundreds of extractions in case of microextraction techniques. The form and volume of these phases can thereby be varied according to individual needs. They can be applied to a solid mechanical support in cases such as SPME or solid-phase dynamic extraction (SPDE), be packed into syringe needles (microextraction in packed syringe or MEPS) and thicker steel capillaries (ITEX), or be simply used as bulk material that is cut into pieces and used freely together with almost any desired sample agitation option as in case of the Sorb-Star (IMT GmbH, Vohenstrauß Germany)[2].

Besides the basic decision whether to sample from the sample solution directly or from the headspace, both possibilities enable the use of static and dynamic methods. While there is no "official" nomenclature on which methods may be referred to as "dynamic", this term is often used for methods that are based on a constant purging of either the sample solution or headspace by a stream of gas, that is subsequently depleted of the purged analytes by a sorbent trap[9,10]. Static sampling methods on the other hand, abstract an aliquot of the sample solution or headspace for direct injection into the injector of the gas chromatograph (GC). Static enrichment methods such as SPME introduce an additional phase into the sample vessel for ab- or adsorbing analytes from either solution or headspace.

In case of extraction from the headspace, analytes would then transfer from the sample solution into the headspace in an attempt to re-establish the concentration equilibrium of analyte concentrations between the liquid and the gaseous phase. This process proceeds until sample solution, sample headspace and sorption phase are fully equilibrated according to the individual analytes distribution constants. Therefore, enrichment-based methods usually extract larger amounts of analytes from a sample compared to static sampling techniques, at the cost of an increased sampling time[11,9,12].

It may be controversially debated, where the line between enrichment-based and dynamic techniques has to be drawn. Especially systems such as ITEX, which will be explained later, also work via a stream of gas that is depleting the sample headspace from analytes which are in parallel enriched on a sorbent bed. The only difference to "classical" dynamic techniques such as purge & trap (P&T) would then be the technical nature of this stream of gas. It is constant in the latter "flow-through" or stripping case and intervallic for ITEX, which is based on a closed system[13,8].

One could propose that the common basic feature of such techniques - fostered extraction by a stream of gas - is more profound than the technical question of how this stream is realized in certain systems. Other examples for this logical discrepancy are trap enrichment systems, which are also a "flow-through" technique but sometimes in an intervallic manner. These systems are also capable of practically exhaustive extraction of analytes by a stream of gas that is directed through the sample vial. By strict application of the traditional definition of dynamic techniques, trap sampling would rather be described as an enrichment sampling system similar to SPME. Those two techniques are, however, fundamentally different in their mode of action and effectiveness.

Chapter 4 of this work contains a detailed evaluation of two techniques (trap enrichment & ITEX) which would traditionally be described as enrichment based. As will be discussed in that chapter, possible differences in extraction yields between these two techniques and, e.g., P&T are practically negligible (depending on the application). It might therefore be more meaningful to differentiate between solventless sample preparation techniques according to the schematic presented in Figure 2, with the fundamental method classes being: Static sampling, static enrichment and dynamic enrichment.

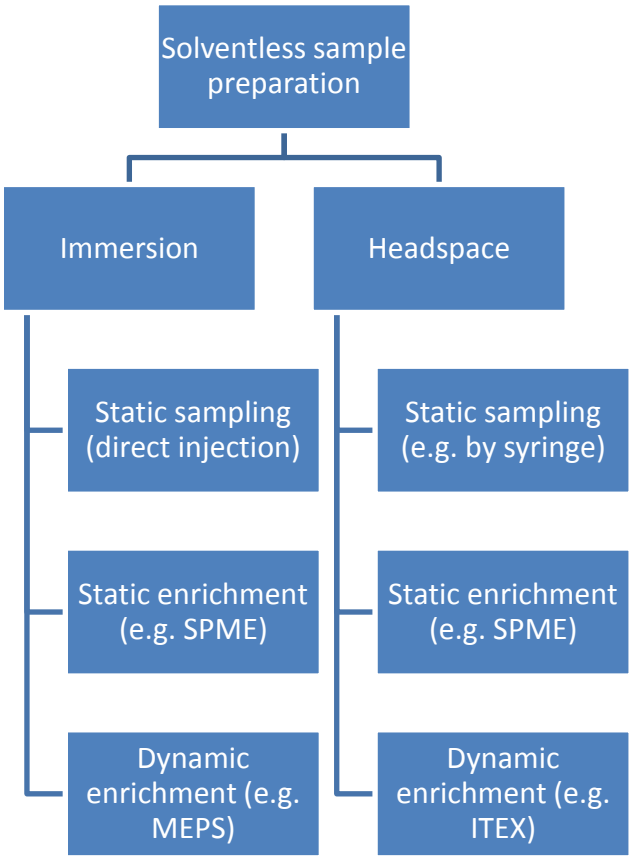

Figure 2: Proposed schematic for a useful differentiation between solventless sample preparation techniques.

1.3 Headspace sampling techniques

As indicated above, microextraction options cannot only be used to extract analytes directly from the sample solution but also from its headspace as depicted in Figure 3. This distinct difference has to be taken into account during initial estimation of, e.g., necessary extraction times. In case of direct immersion sampling, a simple two-phase system is present, resulting in an exchange of analytes between the sample solution and the sorption phase (a)). Should the sorption phase be placed in the sample headspace, a three-phase system has to be considered, in which analytes are primarily exchanged between the sample solution and its headspace (b)) and secondarily also between the headspace and the sorption phase (c)).

Typically, sorption phase selection is carried out in a way that maximizes the transfer of the target analytes into this phase (c)), so that it effectively acts as a sink in this three-phase system[1].

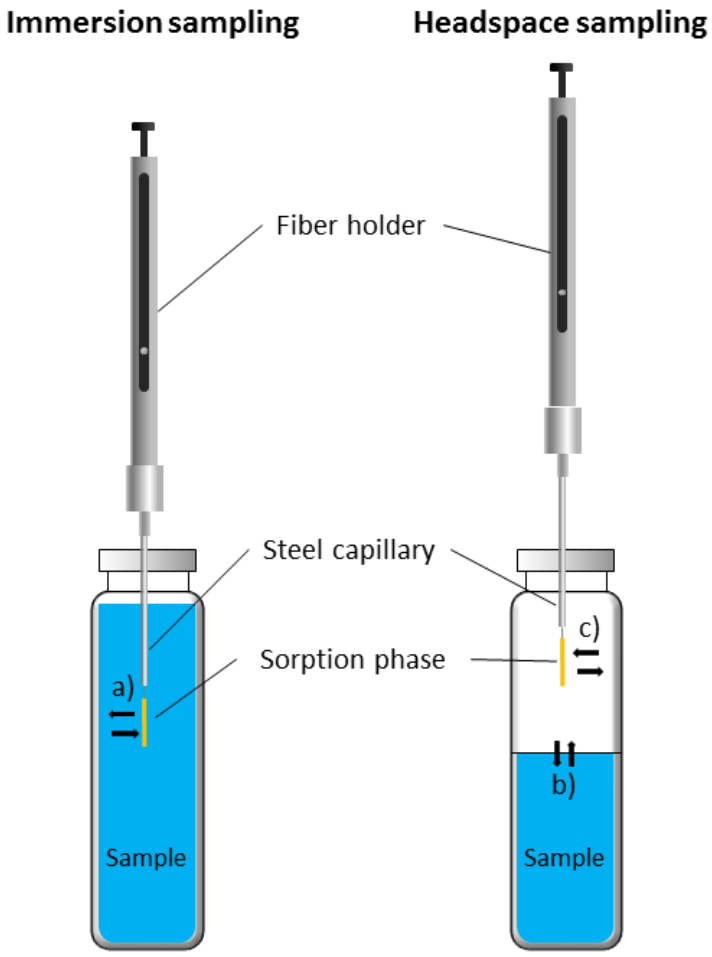

Figure 3: Schematics of immersion and headspace sampling shown for SPME

The transfer of analytes from the sample headspace into or onto the polymeric phase (c)) is thereby significantly faster compared to the similar process occurring during direct immersive sampling (a)). The reason behind this is that the latter is impeded by a diffusion boundary layer that is formed around the, e.g., SPME extraction phase in a solution. In this layer, only diffusive transport is possible in contrast to the turbulent solution around it[7].

While this layer is also present in headspace sampling, and while it can be reduced in thickness by effective agitation of the sample solution, it is still always more pronounced in a solution compared to a gaseous phase due to the much lower diffusion coefficients of analytes in a condensed phase compared to the gas phase. Despite the additional phase transition in the three phase system of headspace sampling, these two transitions (solution to headspace (b)) and headspace to sorption phase (c))) are therefore faster than the single transition (solution to extraction phase) during immersive sampling.

Additionally, the headspace acts as a potential barrier for undesired matrix constituents that may contaminate the sorption phase during immersion, impeding its re-usability. In the end, indirect sampling of analytes from the headspace is therefore typically preferred over immersive extraction so that the latter is only carried out if the volatility of the desired analytes is insufficient[4,12,14].

For these reasons, headspace analysis is very popular. In addition, practical execution is simple and instrumental demands are few: The only necessities for this type of analysis are selection of proper sample vials, -caps and -septa that in combination enable an air-tight sealing of the samples during storage, equilibration and extraction and a gas-tight syringe for transferring headspace gas to the GC injector[4].

If this cannot be assured, the sample headspace would exchange with the ambient lab air, resulting in a constant loss of analytes and highly inconsistent results.

Since headspace analysis (HS) is a well-established concept and widely used nowadays, a multitude of HS methods has become available to the potential users[15]. The individual advantages and drawbacks of the different options are thereby only vaguely apparent due to their biased presentation by their manufacturers. Comparison studies have so far only been carried out in a very limited fashion such as static headspace analysis via syringe (SHS) versus headspace-SPME (HS-SPME)[16].

Headspace sampling via syringe or SPME is a static approach, during which only the sample solution is agitated while the headspace gas is not directly moved. Another possibility is however to foster the phase transfer of analytes from the sample headspace to the extraction phase by actively cycling the headspace gas over or through the sorption phase material[15]. Examples for this are SPDE, ITEX and trap sampling systems.

The ITEX system for instance is based on a packed sorption phase with a volume of approx. 160 µL over which the headspace gas is cycled repeatedly by a gas-tight syringe. The method (newest version depicted in Figure 4) was subject to thorough evaluation during a PhD thesis[2] preceding this work. It can be seen as an improved variant of the classical solid-phase dynamic extraction (SPDE)[13], replacing the internal coating of a syringe in case of the latter technique with a packed sorbent phase with significantly larger phase volume. In its newest version it is called ITEX DHS (dynamic headspace) and in contrast to the former ITEX 2 it now features an active cooling by a fan, enabling faster overall analysis times due to an accelerated cooling of the sorption phase after thermal desorption and cleanup. Furthermore, it does not require anymore an external, additional power supply, is available as a standard PAL3 tool and is therefore easier to install and exchange between instruments. The development process that led to the development of ITEX out of SPDE is thereby comparable to the one that led to PAL SPME Arrow out of the classical SPME fiber.

Figure 4: The newest version of in-tube extraction instrumentation for the PAL3 sampling platform: The ITEX DHS tool with active cooling for the sorption phase. Picture kindly provided by CTC Analytics AG

Trap systems function slightly different in this regard, as the headspace gas is flushed through them exclusively in one direction. Since this would otherwise promote analyte losses due to breakthrough, sorbent-filled volumes are significantly larger in this case with several mL[4].

For full efficiency, microextraction methods require initial evaluation for the specific analytical demand. Parameters are, e.g., the extraction time (correlating to the thickness of the extraction phase[17]) and stirring velocities. An overview of phase volumes of microextraction techniques is given in Figure 5.

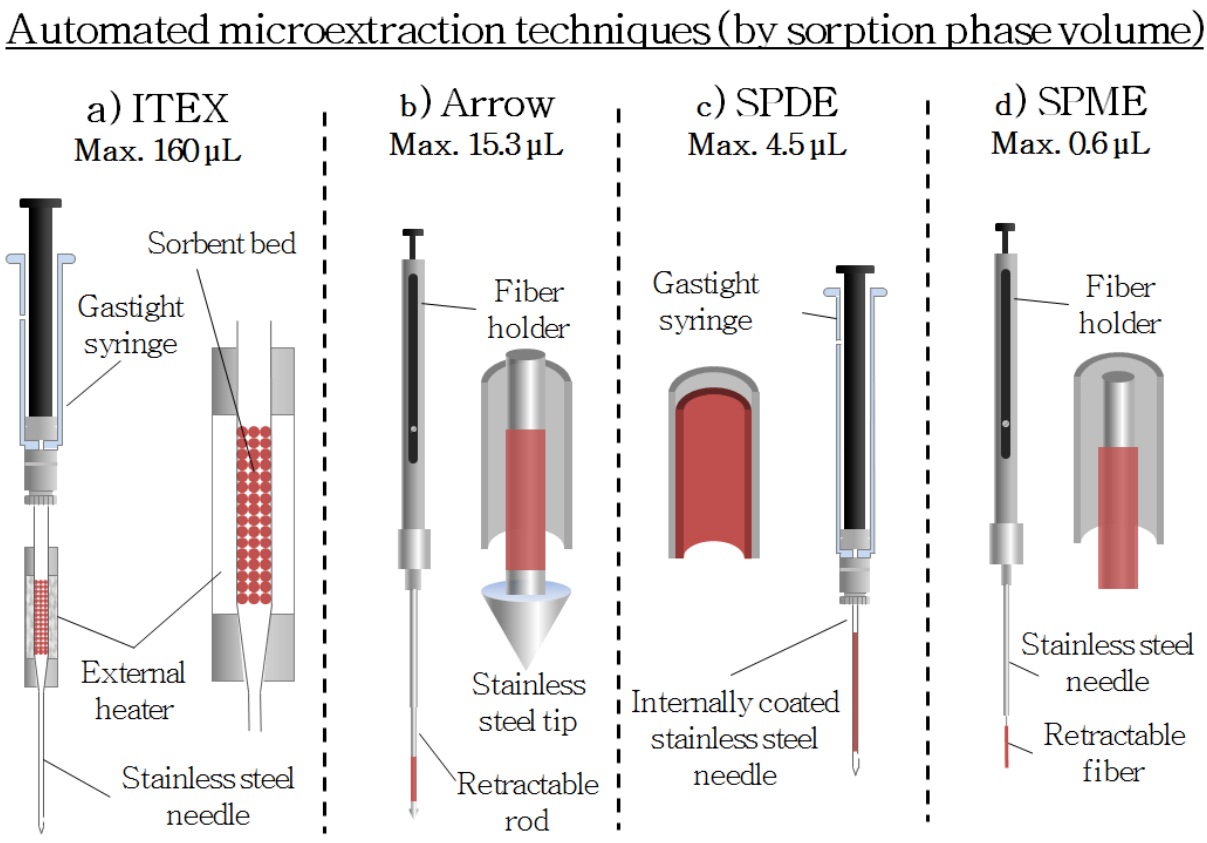

Figure 5: Schematic comparison of four microextraction techniques, organized by their maximum sorption phase volumes: ITEX, PAL SPME Arrow, SPDE and SPME

1.4 Solid-phase microextraction

Solid-phase microextraction is clearly the most wide-spread and well-known microextraction technique of today. It was introduced by Belardi and Pawliszyn in 1989[3] and consists of a, e.g., gauge 23 stainless steel capillary containing a metal wire carrying a fused silica fiber coated with a typically organic sorption phase such as PDMS[18]. The operational principle is thereby as follows: The outer capillary of the device penetrates the septum of a sample vial and the inner wire is lowered. This exposes the organic sorption phase either directly to the liquid sample or to its headspace. Extraction of the analytes occurs, which partition between this extraction phase, the sample liquid and the sample headspace depending on their individual distribution constants. Due to the often high partition constants between extraction phase and either liquid or headspace, under equilibrium a significant fraction of the analytes is extracted despite its comparatively small volume, combining enrichment and matrix separation of the analytes into one step[4].

Subsequent thermal desorption occurs likewise with the outer capillary penetrating through the septum of the injector and exposure of the sorption phase inside the injector liner. The latter should thereby be as narrow as possible in order to ensure maximal linear velocity of carrier gas around the sorption phase, leading to proper peak shapes without the necessity of a large split ratio. This can be fostered further by a rather high temperature of the injector port for quick desorption of analytes from the polymeric sorption phase. It should however be taken into account that such phases also tend to bleed and degrade at higher temperatures such as PDMS releasing cyclosiloxanes. A suitable value for desorption of PDMS is between 250 and 270°C depending on the volatility of the measured analytes.

SPME offers a high potential for sample pretreatment due to its simplicity, but also suffers from two major drawbacks that have not yet been effectively remediated: The mechanical fragility of the fibers and its very limited sorption phase volumes of approx. 0.6 µL[4,7].

Several attempts were made in order to overcome these drawbacks. However, effective remediation of one drawback was so far accompanied by the loss of at least one of the advantages of the SPME concept. An example for this is the stir bar sorptive extraction (SBSE), which enlarges the sorption phase volume at the cost of full automation[19-22].

Another recent alternative is the PAL SPME Arrow that has been evaluated within this work. It retains the advantages of the classical fiber and combines them with increased mechanical robustness and full automation[7]. It is presented and discussed in detail within chapter 2 of this thesis.

1.5 Solid-phase extraction automatization

Solid-phase extraction (SPE) is based on an, e.g., aqueous sample containing target analytes, being passed through a packed sorption phase. The latter phase has functional groups enabling selective retention of the analytes, while the original solvent - in this case water - and undesired matrix constituents pass mostly unretained. Afterwards, residual water is removed from the cartridges as exhaustive as possible, before the retained analytes are eluted by a small volume of an appropriate organic solvent. The latter can then directly be injected into the chromatographic system or be subject to further purification and concentration steps[23,4].

The automation of SPE procedures is not a novel concept [23,24]. So far, however, it typically requires the use of additional instrumentation aside of the autosampler, which can hardly be interfaced directly to a GC. Instead, the resulting eluates usually have to be transferred to the GC manually, so that this approach not only still requires considerable time but also additional footprint inside the laboratory for the dedicated SPE sampling instrumentation[25,26]. True on-line SPE couplings were so far mostly available for liquid-chromatography (LC) applications, for which the SPE process is, e.g., realized via an additional column which is packed with the SPE material and integrated into the flow system of the LC apparatus via automated valves[27,28]. Adaptions of similar systems for gas chromatography were so far limited to custom-made implementations such as the system of van der Hoff et al.[29] in which the eluate is aliquoted in a sample loop prior to being introduced into the GC injector.

A notable exception to this general trend is microextraction by packed sorbent (MEPS), which is based on liquid handling syringes that contain packed SPE sorbent, over which the sample liquid can be cycled.

While this solution allows a full automation, the amount of sorption phase is limited to approx. 1 to 2 mg. Furthermore, the sorption phase is re-used multiple times which limits waste but requires the samples to be completely free of any particles that may otherwise clog the sorbent bed. In addition, a potential carryover has to be taken into account so that rinsing volumes of 0.5 mL after each extraction are usual, resulting in a noteworthy consumption of organic solvents[30-34].

An alternative option called ITSP (instrument-top sample preparation) (ITSP Solutions, Inc. Hartwell, USA) provides the possibility for miniaturized SPE (MSPE). This option is entirely automatable via the PAL sampler, without the need of an additional, dedicated instrument. It is based on downscaled (approx. factor 10 compared to classical SPE cartridges with, e.g., 500 mg of sorbent) SPE cartridges that fit into specially designed well plates for the sampler, enabling it to load, transport and elute these cartridges using liquid syringe tools[35]. This downscaling also brings the necessary volumes of organic solvents into ranges that might be small enough (approx. 50 µL) to consider these cartridges a microextraction option as well, with the obvious exception that such solvents cannot be reused in contrast to, e.g., the polymeric sorption phases of SPME. Besides classical SPE processes, ITSP cartridges can also be used for filtration of samples, for example after a dispersive liquid-liquid microextraction (DLLME) of wastewaters or for cleanup of, e.g., QuEChERS (quick easy cheap effective rugged safe) extracts[36].

Similar to PAL SPME Arrow, these cartridges represent a novel solution based on a well-established methodological concept that had to be evaluated in order to assess its analytical potential. Contrary to PAL SPME Arrow however, the extraction phase is smaller compared to the original technique in case of ITSP, yet again the influence on method sensitivity was an important aspect. Furthermore, the automation of the sampling workflow for these new cartridges was not yet available and required thorough programming and evaluation, especially since it was directly coupled to an on-line evaporation and derivatization process, all automated via a single autosampler sequence.

1.6 Scope of this work

The main scope of this work was to develop and validate two new automated sample preparation methods. The first of these techniques is PAL SPME Arrow, a novel option in the field of microextraction techniques that maintains the original concept and the advantages of SPME and augments these with increased mechanical robustness and sorption phase volumes for improved reliability and sensitivity as described in chapters 2 and 3. It was thoroughly evaluated for immersive and headspace extraction of various analytes in order to demonstrate its potential.

The second new technique was based on ITSP, which can be used for fully automated SPE procedures on PAL-type autosamplers as described in chapter 5. Especially the development of a suitable automation program for this option was a major part of the work presented herein. For both PAL SPME Arrow and ITSP, achievable limits of detection as well as measurement repeatability and general reliability were of special interest.

Another part of the presented work evolved around the topic of headspace analysis techniques and aimed at a meaningful comparison of several available techniques.

This comparison focused on repeatability of results, achievable method detection limits and possible extraction yields, and comprised altogether 6 techniques. Results are depicted in chapter 4. Of special interest were thereby the differences between microextraction solutions that are automatable via PAL-type samplers on the one hand and dedicated headspace sampling instruments on the other hand.

2 PAL SPME Arrow - Evaluation of a Novel Solid-Phase Microextraction Device for Freely Dissolved PAHs in Water

This chapter has been partially published in a modified form in [7]

2.1 Introduction

Solid-phase microextraction (SPME), was developed by Belardi and Pawliszyn in 1989[3] and is nowadays the most popular and most frequently used microextraction technique.[14] The reasons for this popularity are its operational simplicity, short extraction times, possibility of a fully automated operation, avoidance of organic solvents[37] as well as its direct and straight-forward thermodesorption into a gas chromatographic system. Furthermore, SPME combines matrix separation of analytes with a concentrating step[20] and can be used for in-situ, in-field and even in-vivo sampling[38,17,14]. However, apart from many advantages, it also has drawbacks, including the limited mechanical robustness of the fiber[39,19,40,41] and the rather small sorption phase volume of the commercially available fibers[19,14,42].

In order to overcome especially the latter disadvantage, the SPME related technique stir bar sorptive extraction (SBSE) was developed. SBSE provides a significantly larger extraction phase in the order of 100 µL compared to about 1 µL with classical SPME, but loses the advantage of full automation, as the SBSE bar has to be recovered from the sample, dried and introduced into a special thermodesorption unit in a manual process.

Recently, a novel SPME related extraction device named PAL SPME Arrow was developed. As the first alternative in this field to be based on a completely redesigned, automatable fiber, it aims on combining the advantages of the classical SPME fiber and the SBSE, while remediating the main inherent disadvantages of these techniques. It is presented in Figure 6 alongside a classical SPME fiber and its properties will be thoroughly discussed in the results and discussion section.

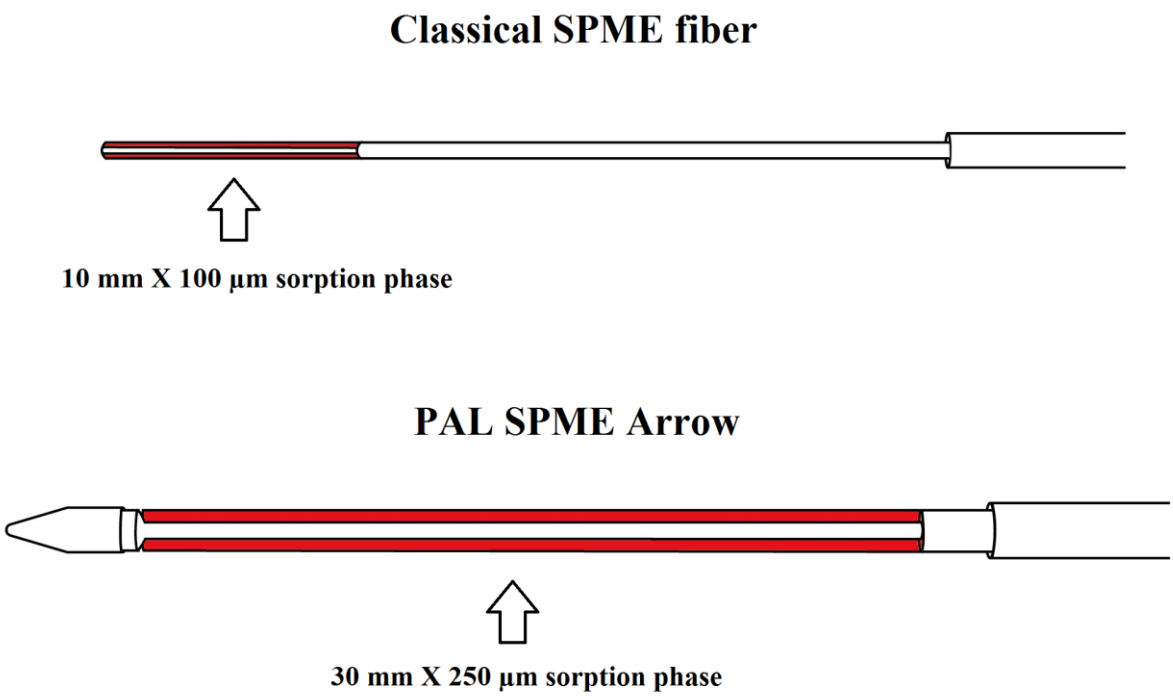

Figure 6: Sketch of a classical SPME fiber and a novel PAL SPME Arrow. The SPME fiber possesses a 100 µm x 10 mm, 0.6µL sorption phase. The PAL SPME Arrow is equipped with a 250 µm x 30 mm, 15.3 µL sorption phase respectively, has a stainless steel inner core with a diameter of 0.4 mm and an overall external diameter of 1.5 mm

Polycyclic aromatic hydrocarbons (PAHs) are abundant environmental contaminants originating both from anthropogenic as well as natural sources, which typically involve incomplete combustion processes such as forest fires or burning of fossil fuels[38,43]. PAHs are also contained in bitumen-related products that are used in various fields of construction, especially due to their hydrophobic properties, which make them a widespread choice for water proofing applications[44]. While the fumes and vapors that originate from production and handling of such materials are already suspected to represent occupational risks in terms of exposure to PAHs[44], the leaching of the latter compounds into runoff water was mostly neglected in the past, often due to insufficient detection limits of the analytical methods[45].

PAHs are known human carcinogens and are metabolically activated inside the cells by Cytochrome P450 enzymes and peroxidases. Thereby they are transformed intro reactive intermediates with the potential of inflicting DNA damage[46].

For this reason and their ubiquitous presence in our environment, PAHs are regulated and constantly monitored priority pollutants[47]. For example, the European Water Framework Directive subsumes all PAHs into one parameter, which is related to the concentration of benzo(a)pyrene as representative compound[48,5].

While the technical specifications underlying these regulations only vaguely state how the challenging threshold values in the pg L-1 range have to be surveilled ("…acceptable level of accuracy and precision…"[48]), it is clear, that classical SPME may have difficulties in fulfilling such analytical demands.

Typical SPME LODs and LOQs for measurements of PAH in water are in the ng/L range, depending on utilized sorption phase and analytical conditions[49]. In this context of increasing analytical demands, PAHs were used as representative and well comparable analytes in order to determine to which extent PAL SPME Arrow surpasses limitations of classical SPME fibers without compromising original SPME advantages.

PDMS was used as common sorption phase material[50], because just like the aforementioned analytes, it enables effective comparison of results with literature.

2.2 Experimental section

Reagents and Materials. Optimization and calibration was carried out by using a PAH standard (SV Calibration Mix #5 / 610 PAH) purchased from Restek (Bellefonte, PA). The standard contains 16 PAHs in methylene chloride at a concentration of 2 g L^{-1}, respectively (Table 1) Analytical grade methanol (KMF Laborchemie, Lohmar, Germany) and lab water from a PURELAB Ultra analytic water purification system (ELGA LabWater, Celle, Germany) were used as solvents for stock, standard and sample preparation. In case of groundwater samples, the water was kindly supplied by LINEG (Kamp-Lintfort, Germany) and filtered through medium dense MN 615 cellulose filters with a thickness of 0.16 mm and a surface weight of 70 g m^{-2}, which were obtained from Macherey-Nagel (Düren, Germany). G200 DD sanded roofing felt according to EN 13969 and EN 14967 was purchased at a Hornbach building supply store (Essen, Germany).

Table 1: Constituents of the EPA PAH calibration mix used for the evaluation of immersive sampling from water with PAL SPME Arrow

Order of elution	Analyte	CAS-Nr.
1	Naphthalene D8	1146-65-2
2	Naphthalene	91-20-3
3	Acenaphthylene	208-96-8
4	Acenaphthene	83-32-9
5	Fluorene	86-73-7
6	Phenanthrene	85-01-8
7	Anthracene	120-12-7
8	Pyrene	129-00-0
9	Fluoroanthene	206-44-0
10	1,2-Benzanthracene	56-55-3
11	Chrysene	218-01-9
12	Benzo(b)fluoroanthene	205-99-2
13	Benzo(k)fluoroanthene	207-08-9
14	Benzo(a)pyrene D12	63466-71-7
15	Benzo(a)pyrene	50-32-8
16	Indeno(1,2,3 cd)pyrene	193-39-5
17	Dibenz(ah)anthracene	53-70-3
18	Benzo(ghi)perylene	191-24-2

Standard solutions and samples. From the PAH calibration mix, a methanolic stock solution with a concentration of 1 mg L^{-1} was prepared and stored in a 20-mL amber screw cap headspace vial, with silicone/PTFE septa and no headspace (BGB Analytik, Boeckten, Switzerland), in the refrigerator at 4°C. From this stock solution, aqueous standard dilutions were prepared and stored in the same manner. Hamilton glass syringes (Hamilton, Bonaduz, Switzerland) and Blaubrand® bulb pipettes (Brand, Wertheim, Germany) were used for stock, dilution standard and sample preparation.

The PDMS tubes which were used as extraction phases for PAL SPME Arrows were also obtained from BGB Analytik.

Roofing felt samples were prepared by cutting the material into pieces of 2 mm x 4 cm (approx. 300 mg) and adding one of these pieces to vials containing 19 mL of lab water. Pieces were deliberately used as a whole since further disintegration would have resulted in a larger total surface area of the material and therefore an overestimation of PAH leaching into water.

A headspace volume of 1 mL was left in each of the sample vials by using only 19 mL of the corresponding type of water plus analyte stock solution or solid sample. A complete filling of the vials would have resulted in the outer capillary of the SPME fiber or PAL SPME Arrow being immersed in water during extraction. This is known to have adverse influence on measurement repeatability since irreproducible amounts of water are aspired into the devices by capillary forces. During subsequent desorption in the injector of the gas chromatograph, this water evaporates to significant volume and changes the pressure conditions of the injection. It disturbs the transfer of analytes to the GC column and may cause contamination of the gas in- and outlets of the injector.

Since PAHs readily adsorb to almost any available surface and are thereby lost to solid-phase extraction processes, it is reasonable to calculate their equilibrium ratios that are adsorbed to the surfaces available in the prepared samples in order to avoid biased results[51]:

Partitioning of analytes into the headspace was calculated[52] as, e.g., 0.16 % for naphthalene, which is the most volatile PAH. Analyte loss due to sorption to glassware was calculated as well[53], with adsorbed analyte fractions of, e.g., 0.3 % in case of pyrene. Sorption of analytes to the PTFE septa of sample vial caps was the strongest influence in this context with an equilibrium value of 3.8 % for pyrene[51].

Therefore 3.8 % can be considered to be the maximum value here, resulting in a total loss of adsorbed analytes below 5 %, which was neglected during the further course of this study. In order to ensure proper sample equilibration prior to measurement series, samples were incubated at room temperature for at least 24 h prior to extraction.

Samples were stirred with self-constructed stir bars prepared from 1.5 x 10 mm iron cylinder bolts enclosed in fused silica.

GC/MS instrumentation and parameters. All analyses were carried out on a Shimadzu GCMS-QP2010 Ultra (Shimadzu Deutschland GmbH, Duisburg, Germany). Thermal desorption of the extracted analytes was carried out using a split/splitless injector, which was set to a temperature of 280°C. The injector was equipped with a Restek (2 mm inner diameter (I.D. x 5 o.d. mm x 95 mm length) splitless liner (BGB Analytik, Boeckten, Switzerland). The thermal desorption time was 5 minutes and after a splitless time of 6 minutes, the split ratio was set to 10:1.

Analyte separation was accomplished on a 30 m x 0.25 mm Rxi®-PAH column (Restek, Bellefonte, PA) with a 0.1 µm film thickness. As carrier gas, Helium 5.0 (Air Liquide, Oberhausen, Germany) with a flow of 1.5 mL min^{-1} was used.

The GC temperature program started with a 5 min standby at 40 °C, followed by a first temperature ramp of 50°C min^{-1} up to 110°C, a second ramp of 5°C min^{-1} to 240°C and a third ramp of 50°C min^{-1} to a final temperature of 320°C, which was maintained for 5 min for cleanup purposes. The transfer line and ion source were both set to 250°C, respectively. Retention times varied between 8.70 min to 49.48 min for naphthalene-d_8 and benzo(ghi)perylene (see Table 2), respectively.

In accordance with literature[54], the chosen chromatographic conditions enabled sufficient separation of all target compounds. A resulting chromatogram is included in the (Figure 1), as well as detailed mass spectrometric detection parameters.

Extraction procedure. Samples were extracted by a PAL RTC autosampler, which was equipped with SPME fibers (100 µm x 10 mm, 0.6 µL) and PAL SPME Arrows (250 µm x 20 mm, 10.2 µL) (all from CTC Analytics AG, Zwingen, Switzerland). The 20 mm long sorption phase was chosen for PAL SPME Arrow to facilitate constant and complete submersion during extraction. 30 mm long fibers, shown in Figure 7 alongside classical SPME fibers, can also be realized for PAL SPME Arrow and offer an even larger sorption phase volume of 15.3 µL. However, depending on the intensity of the agitation and the vortex forming inside the sample liquid (Figure 26), it is difficult to reliably immerse these fibers in their entirety. A complete filling of the sample vials was not carried out here due to the reasons discussed under the standard solutions and samples chapter.

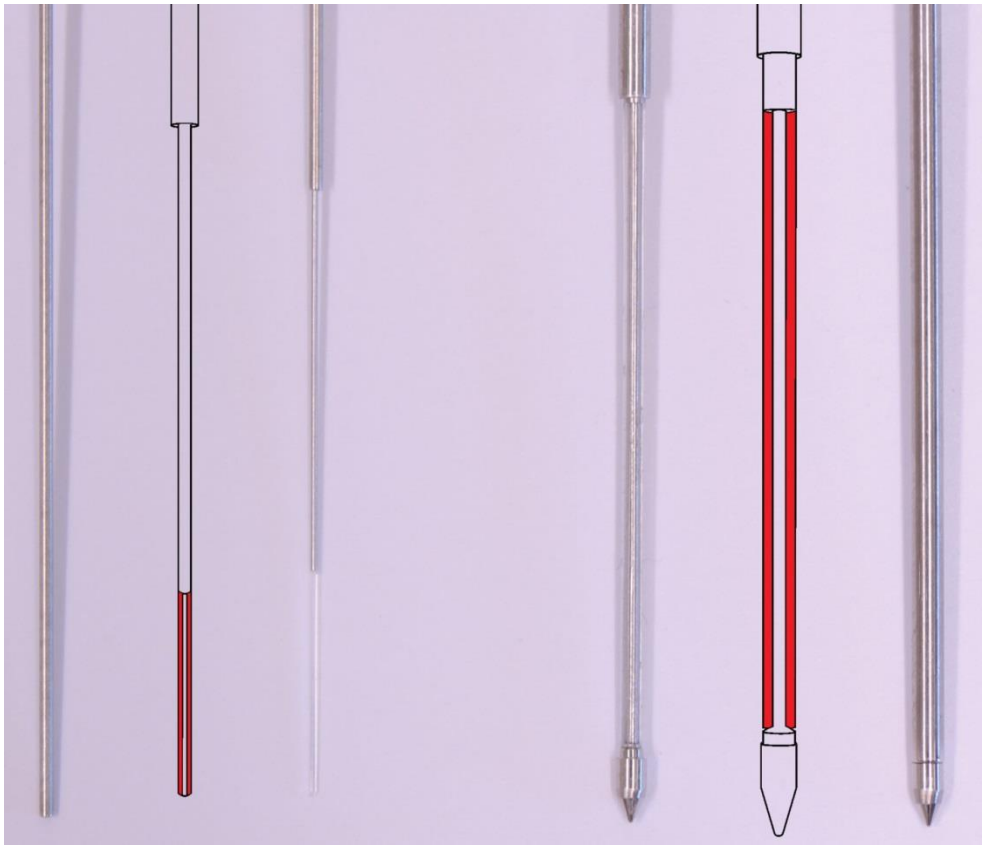

Figure 7: Pictures and sketches of a classical SPME fiber (100 µm x 10 mm PDMS phase, left) and a PAL SPME Arrow (250 µm x 30 mm PDMS phase, right), each depicted in open and closed state respectively.

Due to the larger diameter of PAL SPME Arrow in contrast to traditional SPME fibers, the openings of the PAL tool needle guide, the GC injector and the SPME fiber conditioning station had to be widened to at least 1.7 mm. In Figure 8 the modified components of a split / splitless injector (SSL) of a Thermo Trace GC Ultra are displayed. Further details on this topic will be given in the results section of this chapter.

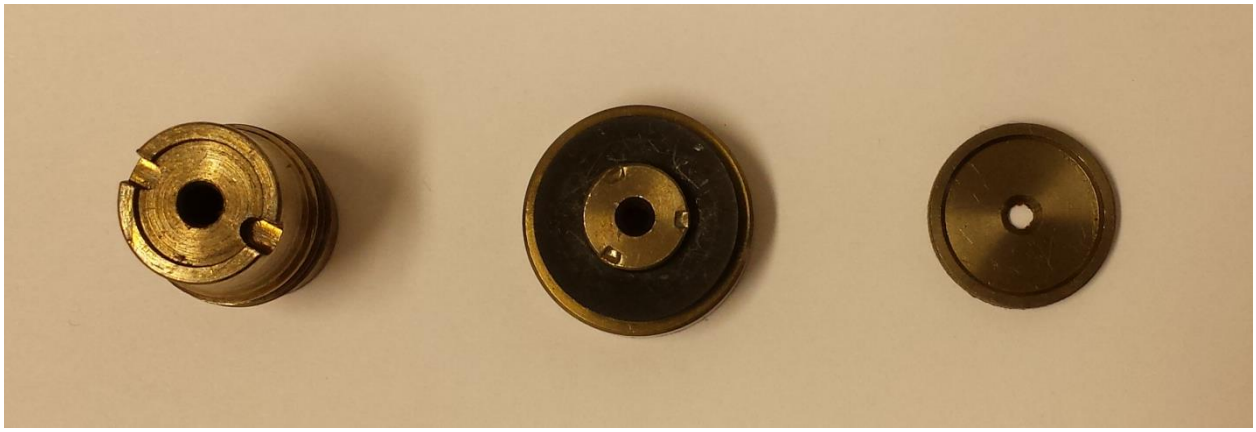

Figure 8: Components of a Thermo Trace GC Ultra SSL injector, modified for use of PAL SPME Arrow

Samples were stored in their tray at room temperature (23°C). Prior to extraction they were transferred to a self-constructed stirring station based on an IKA-Mag RCT basic (IKA-Werke GmbH & CO KG, Staufen, Germany). This alternative is shown in Figure 9 and was developed because the standard PAL agitator proved unsuitable for the use in conjunction with PAL SPME Arrow. This is because any form of agitation that moves the vial and not only the sample liquid transfers it's momentum via the rigid PAL SPME Arrow fiber directly into the sampler, leading to several problems such as disengagement of the screws of the PAL mounting kit. As an interim solution to this problem, agitation solutions with magnetic stir bars were an appropriate option. Since it is however desirable to have an efficient agitation without the need of adding (and recycling) stir bars, the development of an alternative agitation solution was commenced as well. This new solution is currently designated "Heat Ex" and combines an active mechanism that locks the top of the sample vial in place, while the its bottom part is rapidly moved in a double-circular manner ("flower pattern"). This new concept combines efficient mixing and tempering with the absence of stir bars and full compatibility to PAL SPME Arrow. Its development is now complete and it will soon accompany the pending market introduction of PAL SPME Arrow.

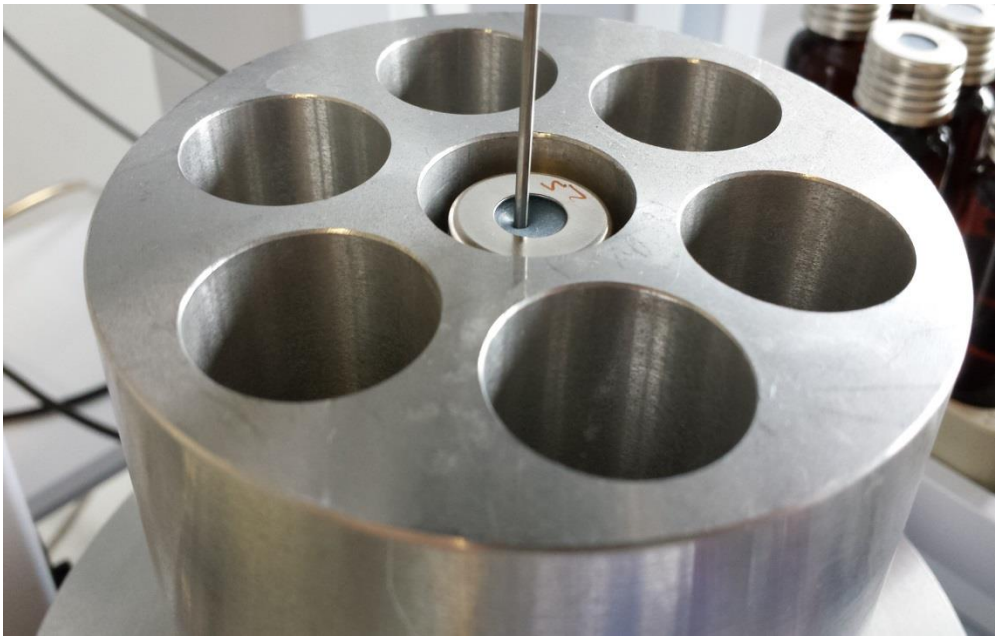

Figure 9: A sample vial in the self-constructed agitation magazine which is situated on top of the IKA-Mag magnetic stirrer/heater combination. The vial septum is currently pierced by a PAL SPME Arrow with a diameter of 1.5 mm

In the self- constructed agitation station, samples were continuously stirred at 1500 rpm and 35°C, first for a temperature pre-equilibration time of 10 min and afterwards during sample extraction. Simultaneous to the first five minutes of sample pre-equilibration time, the SPME fiber or PAL SPME Arrow was preconditioned in the SPME fiber conditioning station at 200°C under a stream of nitrogen 5.0.

After the sample pre-equilibration time, the sample vials' septa were pierced by the fiber and the sorption phase was immersed into the continuously stirred sample for 70 min. The sample vial penetration depth was thereby set to 55 mm, in order to ensure constant and complete immersion of the sorption phase.

Once extraction was completed, the fiber was transferred into the GC injector for thermal desorption at 280°C. Subsequently, it was cleaned for 15 min in the SPME fiber conditioning station at 200°C. The PAL RTC sequence was interlocked so that the subsequent equilibration and extraction was carried out during the GC run of the previous sample in order to reduce overall analysis time.

Chromatographic separation. A Chromatogram of the 16 included PAHs as well as the internal standards at a concentration of 1µg L^{-1} is shown in Figure 10. The separation was accomplished on a 30 m x 0.25 mm Rxi®-PAH column with a 0.1 µm film thickness. Retention times varied between 8.70 min to 49.48 min for naphthalene-d_8 and benzo(ghi)perylene (see Table 2), respectively. In accordance with literature[54], the here chosen chromatographic conditions, in conjunction to the used column, enabled sufficient separation of all target compounds.

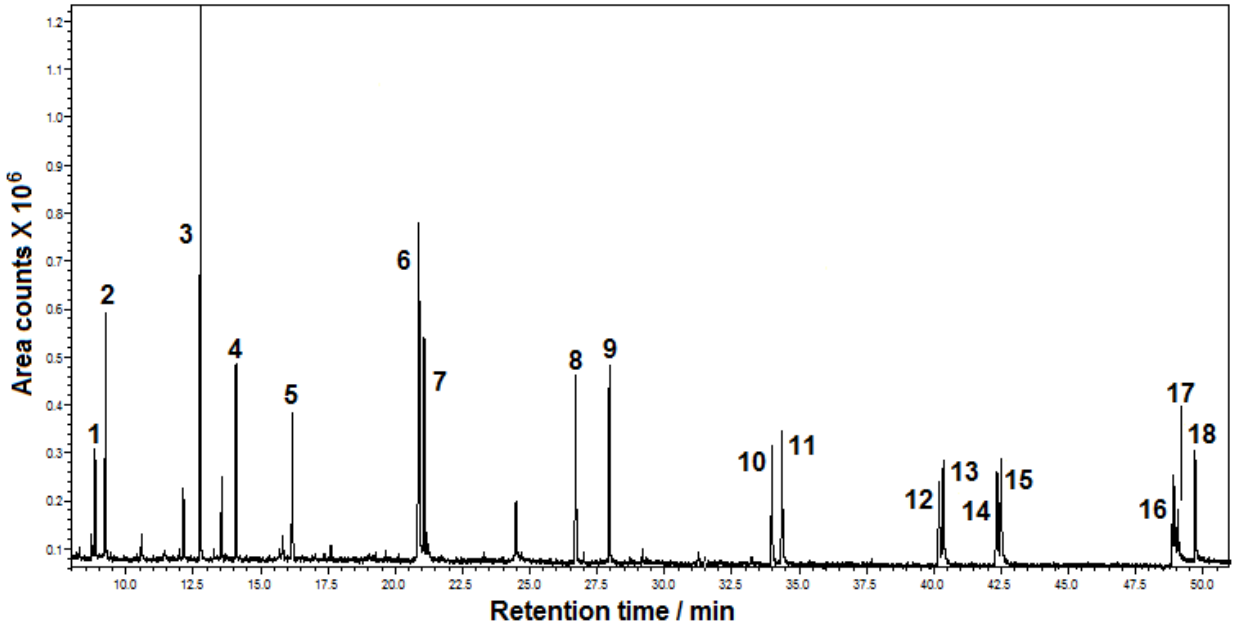

Figure 10: GC/MS chromatogram of standard analytes in full scan mode (m/z range: 50-350) at 1 µg L-1. Target compounds: (1) naphthalene-d8, (2) naphthalene, (3) acenaphthylene, (4) acenaphthene, (5) fluorene, (6) phenanthrene, (7) anthracene, (8) pyrene, (9) fluoroanthene, (10) benz(a)anthracene, (11) chrysene, (12) benzo(b)fluoroanthene, (13) benzo(k)fluoroanthene, (14) benzo(a)pyrene D12 (15) benzo(a)pyrene, (16) indeno(1,2,3-cd)pyrene, (17) dibenz(ah)anthracene, (18) benzo(ghi)perylene

Mass spectrometry. The mass spectrometer was regularly tuned with FC43 (perfluorotributylamine, CAS 311-89-7) (BGB Analytik, Boeckten, Switzerland). Ionization of analytes was performed via electron impact ionization and initial identification of PAHs was carried out in total ion current (TIC) mode in a specified m/z range of 50 to 350. Quantification of the PAHs was then conducted in selected ion monitoring (SIM) mode, using the specific m/z of ions given in Table 2. To reduce the number of simultaneously monitored m/z ratios, nine different SIM segments were used.

In addition, the identification of the analytes was assured by monitoring two specific reference ions at the corresponding molecules' characteristic retention times. Results for naphthalene were normalized via naphthalene-d_8, while all other compounds' results were normalized via the second internal standard benzo(a)pyrene-d_{12}.

Table 2: GC/MS detection parameters for the investigated compounds and internal standards for the evaluation of immersive sampling from water with PAL SPME Arrow

Compound	Retention time	Segment	Quantifier ions (m/z)	Qualifier ions (m/z)
Naphthalene-d_8	8.70	1	136	68, 108
Naphthalene	8.74	1	128	127, 129
Acenaphthylene	13.32	2	152	76, 151
Acenaphthene	13.85	2	154	152, 154
Fluorene	15.92	3	166	82, 166
Phenanthrene	20.60	4	178	76, 89
Anthracene	20.79	4	178	76, 89
Pyrene	26.41	5	228	100, 101
Fluoroanthene	27.66	5	212	100, 101
1,2-Benzanthracene	33.67	6	228	113, 114
Chrysene	34.04	6	228	73, 147
Benzo(b)fluoroanthene	39.82	7	252	125, 126
Benzo(k)fluoroanthene	39.97	7	252	125, 126
Benzo(a)pyrene-d_{12}	41.93	8	264	132, 263
Benzo(a)pyrene	42.10	8	252	125, 126
Indeno(1,2,3 cd)pyrene	48.69	9	276	137, 138
Dibenz(ah)anthracene	48.88	9	278	41, 43
Benzo(ghi)perylene	49.48	9	276	137, 138

2.3 Results and discussion

Fiber development and properties. PAL SPME Arrow is based on a stabilizing stainless steel inner rod that runs continuously through the entire fiber, carrying the cylindrically shaped sorption phase and connecting the upper parts of the device to its solid tip. The tip is shown in Figure 11 and specially designed to allow gentle penetration of septa sealing injectors and sample-vials. This tip also retains the sorption phase, which is attached to the inner rod, and furthermore enables PAL SPME Arrow's capability to enclose the sorption phase during transfer processes. This is an important difference to the traditional SPME fiber, which only allows for the retraction of the latter, with its outer capillary more open to external, potentially adverse influences such as contaminations from ambient air as depicted in Figure 11. The outer capillary rests against the solid tip, resulting in a homogeneously closed fiber since both parts possess the same diameter.

Figure 11: Points of a classical SPME fiber (left) and a 1.5 mm PAL SPME Arrow (right)

Furthermore, an open capillary faces significant resistance during penetration processes, in contrast to a PAL SPME Arrow in its closed state. Classical SPME fibers can cause coring of injector septa due to their open tubular tip[14]. Based on own experiences, exchange of the septa of gas chromatographic systems, which are subject to regular SPME measurements is required after approximately 100 injections to avoid leakages and introduction of septum material into the liner.

Using PAL SPME Arrow, the wear of injector septa was reduced due to the specially designed tip. Despite the enlarged diameter compared to the classical fiber, at least 200 injections without coring, abrasion or leakage are possible.

During the early stages of PAL SPME Arrow development, it was first necessary to identify the most important constructional aspects in order to optimize the general design. The point style of the tip and a smooth transition from this tip to the outer capillary turned out to be the most crucial parameters for a gentle penetration of injector and sample vial septa. An erratic gap between tip and outer capillary present in early prototypes functioned like a grater and successively scraped material from the septum and into the sample or injector below (Figure 12):

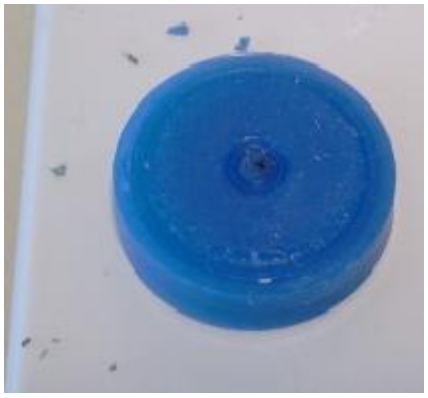

Figure 12: Varian Septum for a Thermo SSL injector with scraped-out material by an early PAL SPME Arrow model that possessed a gap between its tip and outer capillary

This caused two distinct problems: First of all the injector suffered from leaks very soon because only approx. five injections were already enough to completely destroy the septum's sealing abilities as displayed in Figure 13.

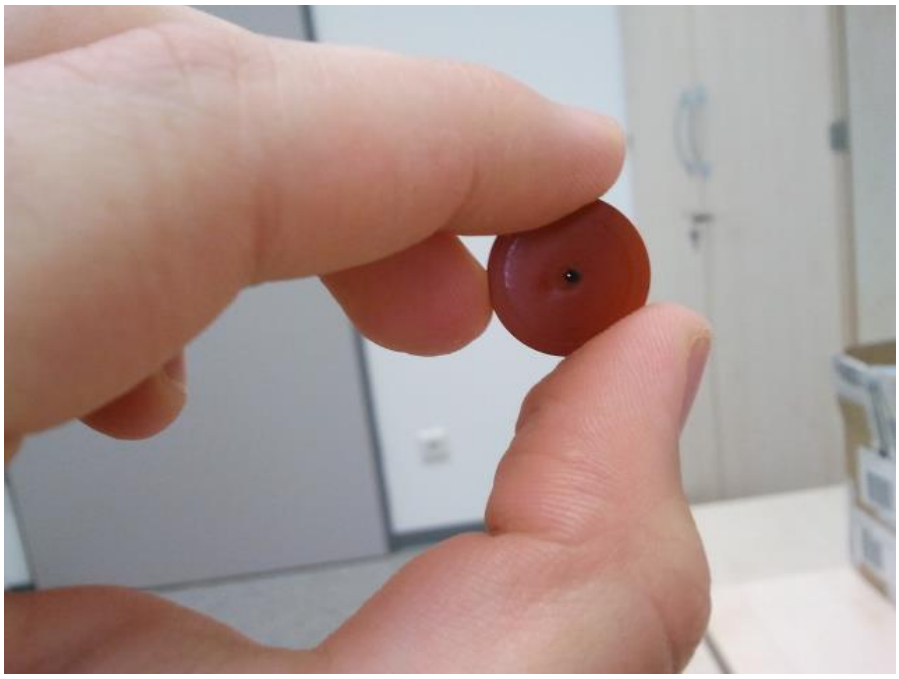

Figure 13: Picture of a BTO septum used in a Termo SSL after a few injections with one of the earlier models of PAL SPME Arrow showing a clear and throughout cavity

Second, the scraped-out material accumulated in the liner of the injector and lead to severe siloxane background in all measurements (e.g., m/z=73). Due to the elevated temperature of the injector, these small pieces of rubber material also partially melted and sticked to the bottom of the injector, what made them hard to remove. In fact, except for the outer casing that is affixed to the top of the GC, the entire injector had to be dismantled regularly in this phase of the work as depicted in Figure 14:

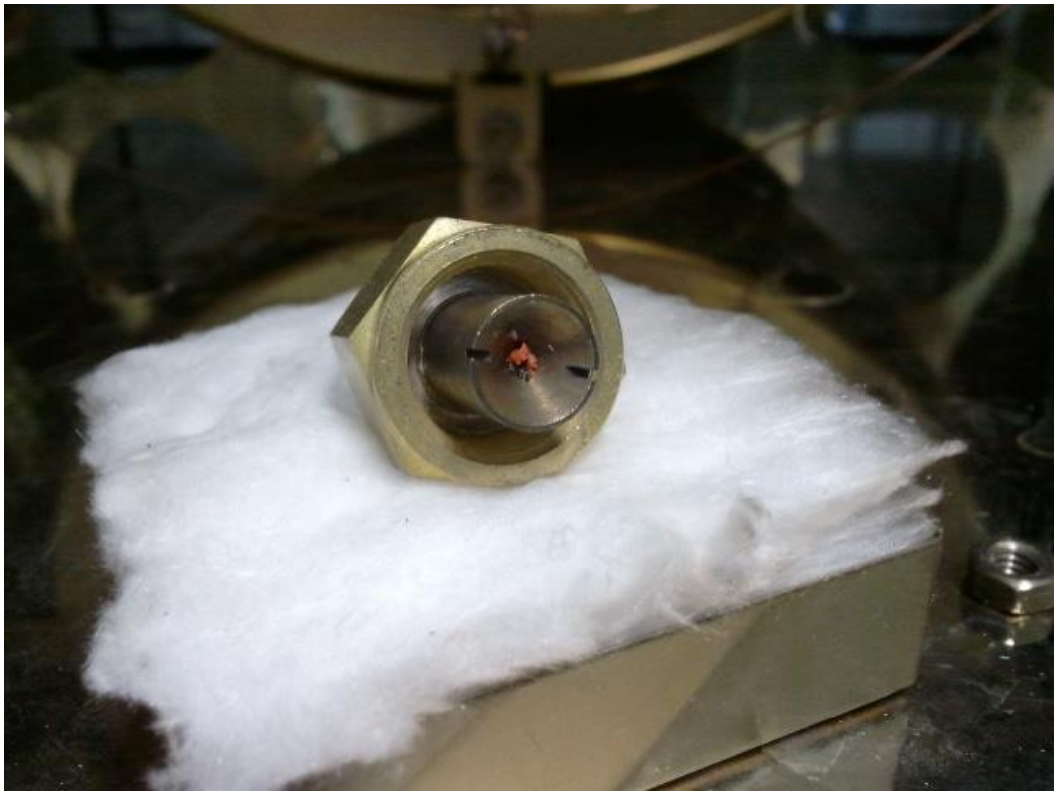

Figure 14: Dismantled base of a Thermo SSL injector with partially melted and converged, scraped pieces of septum material

Sometimes, the scraped pieces of septum material also hit the tip of the GC column, leading to partial blockage and further problems. Therefore, the column also had to be removed regularly from the injector during this stage. After cutting of approx. 10 cm from its beginning, the column was then reinstalled to the cleaned GC injector.

Another problem was the rather rough surface structure of the stainless steel that was used for the construction of the initial PAL SPME Arrow fibers. This structure also abraded material from the septa but to a significantly smaller extent than the scraping that occurred from a gap between tip and outer capillary. Still, after approx. 50 injections, this scraped septum material had formed a visible film on the outer capillaries that was also partially transformed into siloxane contamination with each repetitive injection cycle. A fiber with such a siloxane film is shown in Figure 15. This phenomenon also accounted to limited septa lifetimes as well as a slowly increasing siloxane background over the course of the measurements.

Figure 15: An early PAL SPME Arrow model with an already optimized transition from tip to outer capillary that however still had a rough surface structure leading to the formation of a film consisting of thermally degraded septum material which is visible by a darker coloring of the first few cm from the tip

The solution to this problem was careful polishing of the stainless steel constituting the tips and outer capillaries. After this modification, no further film formation was visible on the fibers and the monitored siloxane backgrounds declined.

Finally, one further aspect to be optimized was the modification of the injector parts. As already mentioned and depicted in Figure 8, the injector parts had to be widened in order to accommodate the enlarged diameter of PAL SPME Arrow. For conservation of the injector septa, however, it was also required to give them a place to be displaced to during penetration by the fiber in order to avoid partial crumbling. As already mentioned, the tip of PAL SPME Arrow is designed to displace the septa in a gentle manner. Therefore, some room had to be created underneath the septum, where the material can temporarily relocate to during the penetration process. For this reason, the supporting metallic piece underneath the septum was not only widened, but also beveled in a way that had to be optimized as well. While an insufficient way of doing so results in an overstressed septum during penetration and therefore in a quick deterioration of it, an exaggerated beveling leads to constant leakage of the injector due to insufficient contact area between the septum base and the underlying metallic piece. Necessary modifications are shown exemplarily for the Shimadzu SPL-2010 injector in Figure 16 alongside the optimized details.

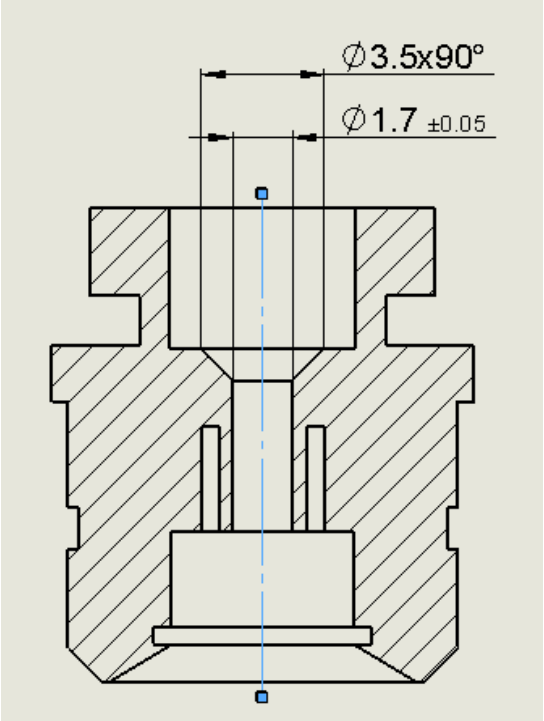

Figure 16: Base component of a Shimadzu SPL-2010 injector, modified for the use of PAL SPME Arrow. All values are given in mm

After completion of this initial development phase, PAL SPME Arrow demonstrated faultless mechanical reliability over the remaining course of these studies. In our experience, classical SPME fibers are more fragile, typically requiring replacement after 100 to 200 injections due to bending of the fibers (Figure 17).

These values seem to be typical and are also encountered in literature[39-41]. Active agitation of the sample vial (instead of the liquid sample via stirring) by the standard PAL agitator may decrease this value even further since the fiber material is weakened by being constantly bent into alternating directions.

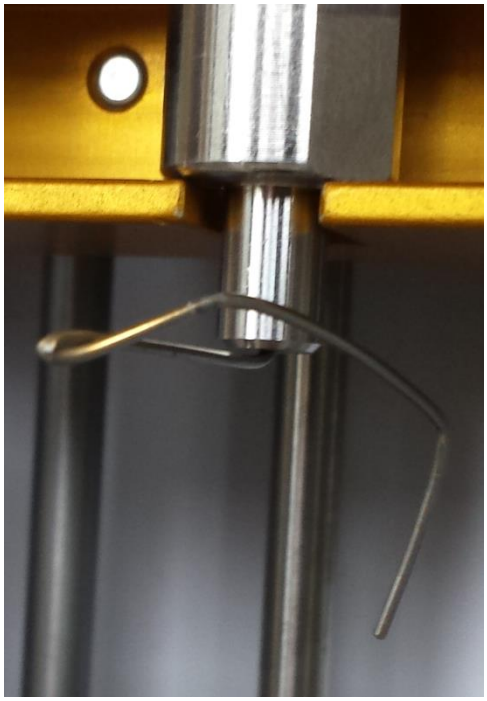

Figure 17: A bent classical SPME fiber after approximately 150 injections

The main reason for this change in mechanical reliability is the increased diameter of the fibers' outer capillary, which is 1.5 mm in contrast to approx. 0.7 mm in case of the classical gauge 23 SPME fiber. In addition, the tip of PAL SPME arrow not only conserves septa during penetration, but thereby also lowers the resistance, that has to be overcome.

PAL SPME Arrow was developed in two different variants, that either possess an outer diameter of (a) 1.5 mm, or (b) 1.15 mm. While both variants support a maximum sorption phase length of 30 mm, the thickness of such phases may be up to 250 µm in case of (a) and 100 µm in case of (b).

Compared to classical SPME fibers, both variants exhibit a significant increase in mechanical robustness and sorption phase dimensions, with a maximum polymer volume of 15.3 µL (25.5 times more volume compared to a classical 100 µm x 10 mm SPME fiber) and a maximum surface area of 84.8 mm^2 for variant (a). The diameter of the inner rod may be varied depending on the desired extraction phase thickness and is usually 0.4 mm for the 250 µm thick phases and 0.5 mm for the 100 µm variants. The resulting outer diameters of the PDMS tubes are 0.9 mm for the 250 µm thick phase variants and 0.7 mm for the 100 µm thick phase variants.

Extraction optimization. In general, PAL SPME Arrow and classical SPME fibers require the same optimization procedure. For the here applied direct immersion (DI) extraction, the important optimization steps are evaluation of extraction time and stirring velocity.[17]

In Figure 18, the influence of stirring rate and extraction time are shown exemplarily for four of the sixteen EPA PAHs, with achieved results confirming expectations according to literature[49,18,14]:

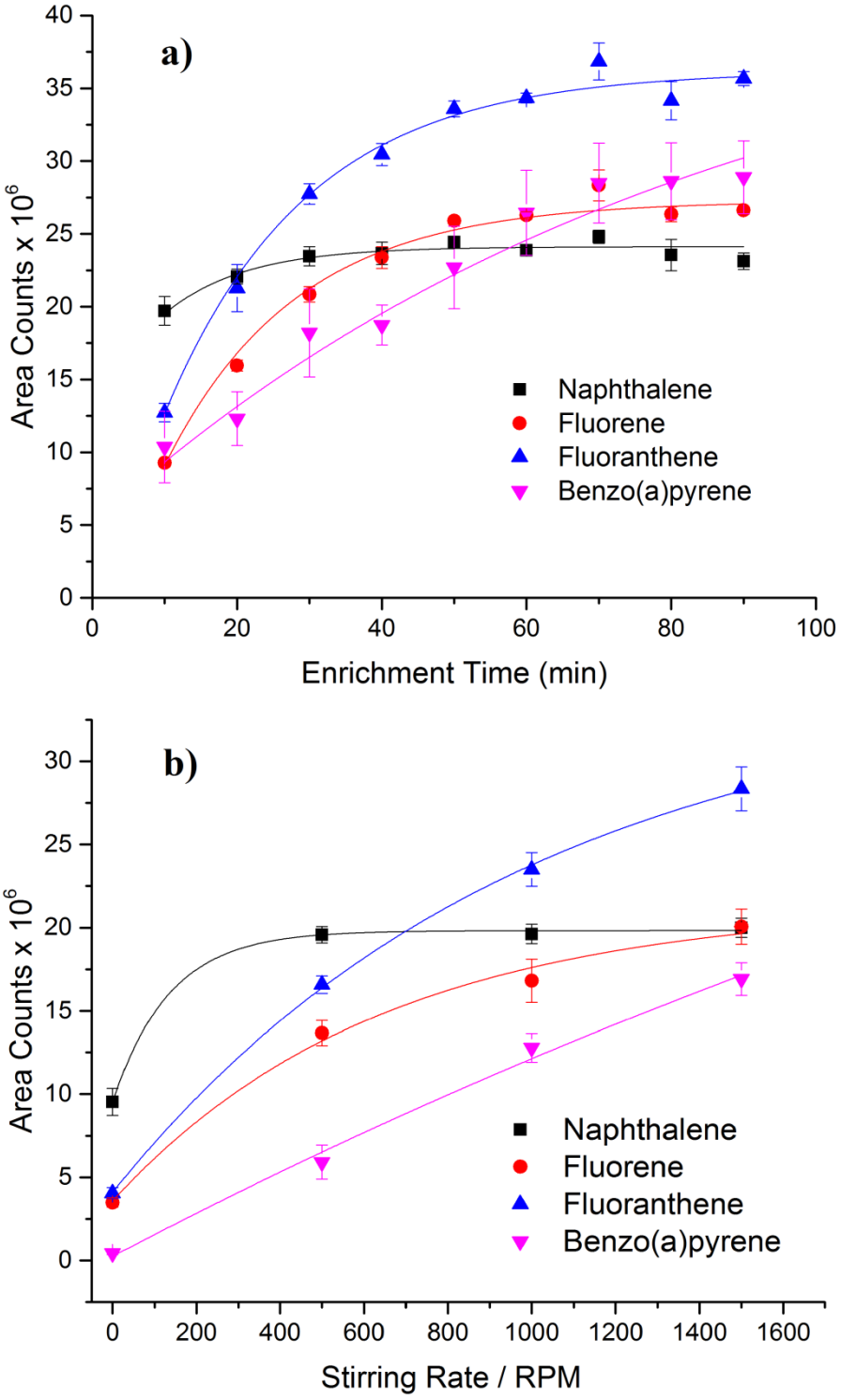

Figure 18: Extraction optimization measurements for PAL SPME Arrow extractions of PAH from aqueous solutions: a) Extraction time (stirring rate kept at 1500 RPM), b) stirring rate (extraction time kept at 70 min). All samples contained 500 ng L-1 of PAHs and were extracted by a PAL SPME Arrow with a PDMS sorption phase (250 µm x 20 mm, 10.2 µL). Exponential trend lines were added via Origin Pro 2015

For the optimized PAL SPME Arrow method, an extraction time of 70 minutes was chosen. Apparently this technique represents a reasonable compromise in this context. Classical SPME fibers, typically require approx. 30 minutes[49] of extraction time in order to reach an equilibrium state and alternative extraction techniques with larger sorption phases such as SBSE may require timeframes of up to 14 hours[55].

In Figure 18 b) the influence of the stirring rate between 0 and 1500 rounds per minute (rpm) is shown. In accordance with the SPME extraction theory,[14] an increased stirring rate leads to a higher mass transfer in the system, since the diffusion layer around the fiber coating is minimized and thus the equilibrium is attained faster. For the optimized method the maximum possible stirring rate of 1500 rpm was used.

Since the typical behavior of decreasing extraction yields at higher temperatures caused by smaller partition coefficients of the analytes between the extraction phase and the sample matrix[14] could be observed in our preliminary measurements as well, the lowest possible temperature of 35°C was used for all sample extractions. (Figure 19) shows exemplary optimization results for anthracene and a good linear correlation for the declining signal intensities (peak areas) with increasing extraction temperatures.

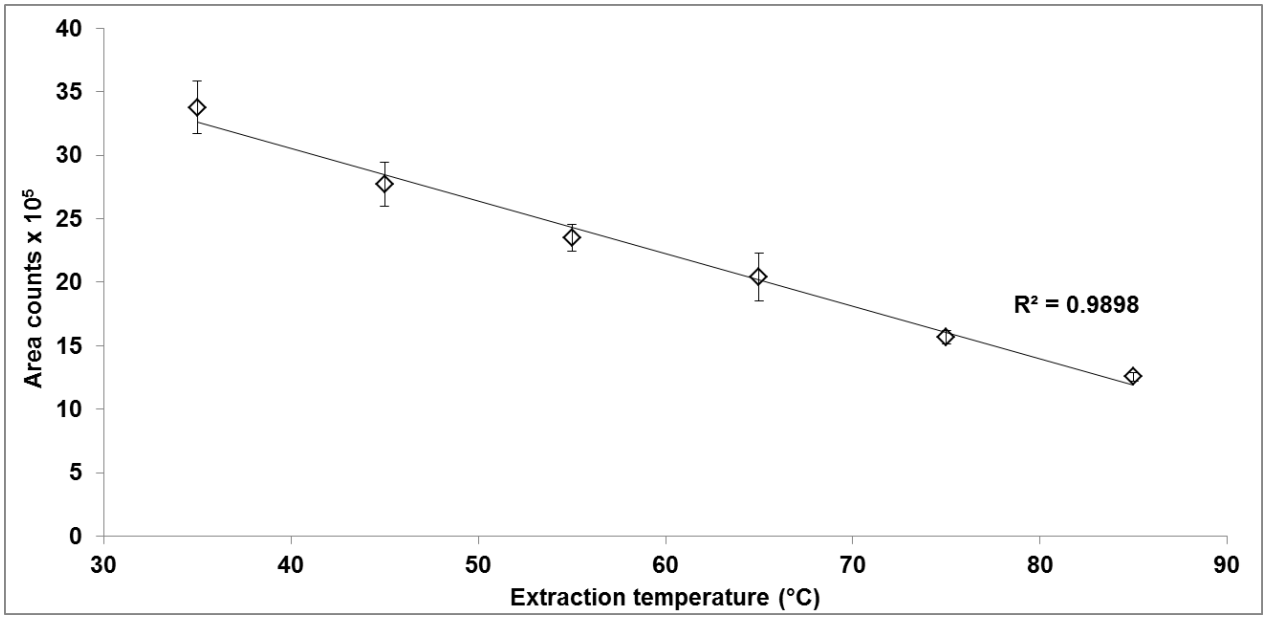

Figure 19: Influence of extraction temperature on anthracene peak areas determined at a concentration of 100 ng L-1 using a PAL SPME Arrow with a PDMS sorption phase (250 µm x 20 mm, 10.2 µL) including a linear trend line with correlation coefficient

Extraction efficiency. To determine the effects of the enlarged sorption phases in case of PAL SPME Arrow, a comparison with classical SPME fibers was performed. Prior to sample measurements, theoretically extracted analyte amounts were calculated with equation (1)[18]:

$$m_f = \frac{K_{fs} V_f V_s c_0}{K_{fs} V_f + V_s} \quad (1)$$

Where m_f is the extracted mass of analyte in the polymeric sorption phase under equilibrium conditions, V_f and V_s are the volumes of the polymer and the aqueous sample, respectively. The initial amount of each analyte present in the aqueous samples with a volume of 19 mL and an initial analyte concentration (c_0) of 10 ng L^{-1} was 190 pg.

The distribution constants K_{fs} for the analytes' phase transition from the aqueous solution into the PDMS sorption phase were calculated from literature parameters[56] and equation (2), yielding the results included in Table 2. The letters *E*,*S*,*A*,*B* and *V* thereby denote the solute descriptors according to the Abraham model for excess molar refraction, dipolarity/polarizability, overall hydrogen bond acidity, overall hydrogen bond basicity and McGowan volume respectively[57].

$$\log K_{fs} = 0.246 + 0.568E - 1.305S - 2.565A - 3.928B + 3.573V \quad (2)$$

Table 3: Calculated log Kfs and mf values for ten exemplary PAHs included in this work, determined for a SPME fiber (100 µm x 10 mm, 0.6 µL), a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL) and an SBSE bar (500 µm x 20 mm, 47 µL) for a c0 of 10 ng L-1, sorted by ascending log Kfs value, based on solute descriptors from literature[26]

Compound	log K_{fs}	m_f (SPME fiber) (pg)	m_f (PAL SPME Arrow) (pg)	m_f (SBSE bar) (pg)	Ratio of extracted masses PAL SPME Arrow vs. SPME fiber / SBSE bar vs. PAL SPME Arrow
Naphthalene	2.8991	4.6	56.7	125.8	12.2 / 2.2
Acenaphthene	3.4196	14.6	111.2	164.7	7.6 / 1.5
Fluorene	3.6313	22.6	132.4	173.6	5.9 / 1.3
Anthracene	3.8933	37.6	153.5	180.7	4.1 / 1.2
Fluoranthene	4.2939	72.8	173.6	186.2	2.4 / 1.1
1,2-Benzanthracene	4.9443	139.7	186.1	189.1	1.3 / 1.0
Benzo(a)pyrene	4.9744	142.2	186.3	189.2	1.3 / 1.0
Benzo(b)fluoroanthene	5.0941	151.4	189.2	189.4	1.2 / 1.0
Benzo(ghi)perylene	5.6407	177.2	187.9	189.8	1.1 / 1.0
Dibenz(a,h)anthracene	5.9609	183.6	189.6	189.9	1.0 / 1.0

According to Table 3, PAL SPME Arrows can be expected to exhibit improved extraction yields when compared to classical SPME fibers with a ratio of up to 12.2 for PAHs. In case of the SBSE bars, the further improvement in relation to PAL SPME Arrow has a ratio of up to 2.2. Especially for molecularly larger compounds with a log K_{fs} of approx. 5 or larger, differences in extraction efficiency between PAL SPME Arrow and SBSE are negligible. Obviously, the effect of a further increase in sorption phase dimensions peaks in the range where PAL SPME Arrow is situated. The critical relation here is the phase ratio between sample and sorption phase. While these results were calculated for 20-mL vials, the SBSE technique is probably better suited for analysis of larger sample volumes, which are however less straightforward to automate.

In order to evaluate these calculated values, the depletion SPME method[58] was used to determine the extracted percentages of analytes out of a sample with an initial concentration of 50 ng L^{-1} for a single extraction. The latter was either carried out by a classical SPME fiber (100 µm x 10 mm, 0.6 µL) or a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL). This method is based on performing depletion extractions by extracting and measuring samples multiple times. The declining, logarithmical peak areas are then plotted against the number of consecutive extractions, yielding a linear regression, whose slope *b* then enables calculation of the extraction ratio *E* from log(1-*E*)[58].

The results of these measurements can be seen in Table 4 and are in good agreement with literature[19], as well as the previously calculated values in Table 3. This is also visible when plotting calculated against measured results with a linear trend line. An example for such plots can be found in Figure 20. These measurements were also carried out for the largest available PAL SPME Arrow sorption phase variant (250 µm x 30 mm, 15.3 µL) and the results are included in Table 5.

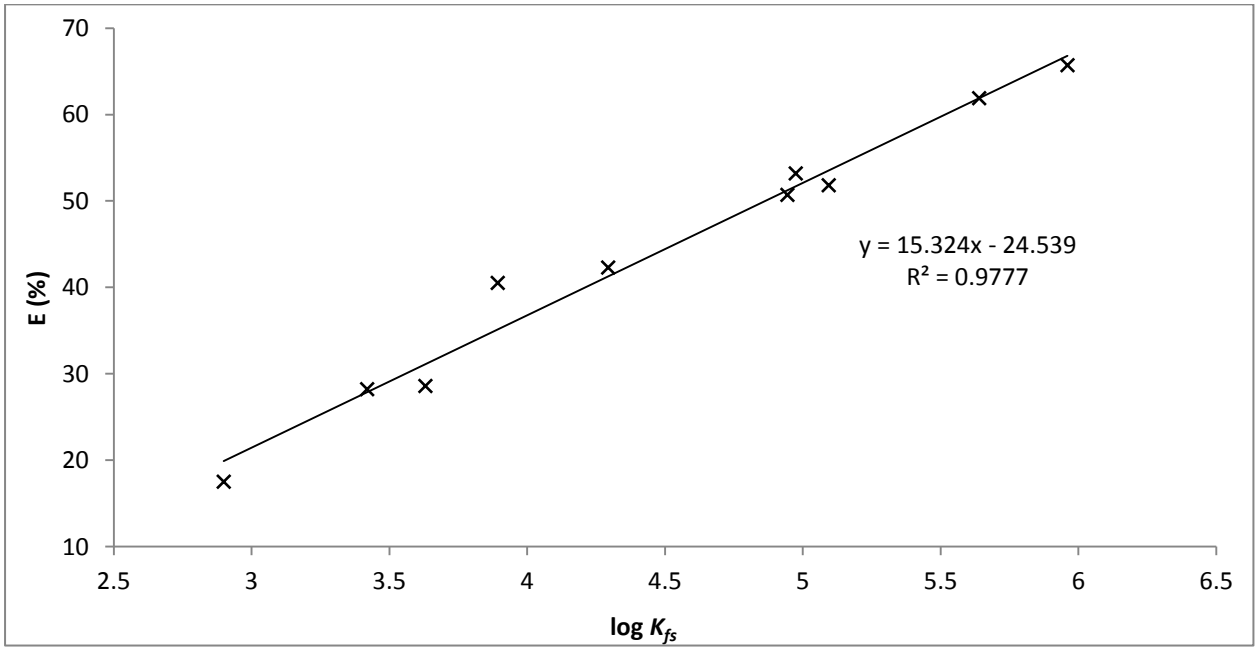

Figure 20: Plot of the calculated log Kfs values and experimentally determined extracted percentages E (%) including a linear trend line

Table 4: Slopes, correlation coefficients and extracted percentages of the performed depletion experiments according to Zimmermann et al.[28] for samples containing 19 mL of water and an initial concentration of 50 ng L-1 PAHs for the first extraction by a classic SPME fiber (100 µm x 10 mm, 0.6 µL) and a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL)

Compound	SPME fiber			PAL SPME Arrow		
	Slope	R^2	E (%)	Slope	R^2	E (%)
Naphthalene	-0.023	0.9903	5.2	-0.084	0.9915	17.5
Acenaphthylene	-0.028	0.9842	6.2	-0.134	0.9980	26.6
Acenaphthene	-0.041	0.9946	9.0	-0.144	0.9907	28.2
Fluorene	-0.050	0.9927	10.9	-0.146	0.9902	28.6
Phenanthrene	-0.060	0.9972	12.9	-0.163	0.9959	31.4
Anthracene	-0.071	0.9930	15.1	-0.225	0.9943	40.5
Pyrene	-0.097	0.9956	20.1	-0.254	0.9993	44.3
Fluoroanthene	-0.096	0.9972	19.9	-0.239	0.9996	42.3
1,2-Benzanthracene	-0.137	0.9925	27.1	-0.307	0.9946	50.7
Chrysene	-0.071	0.9235	15.1	-0.278	0.9871	43.4
Benzo(b)fluoroanthene	-0.172	0.9938	32.7	-0.317	0.9964	51.8
Benzo(k)fluoroanthene	-0.176	0.9878	33.4	-0.412	0.9937	61.3
Benzo(a)pyrene	-0.156	0.9958	30.2	-0.330	0.9854	53.2
Indeno(1,2,3 cd)pyrene	-0.159	0.9845	30.7	-0.367	0.9897	57.0
Dibenz(ah)anthracene	-0.142	0.9926	27.8	-0.465	0.9994	65.7
Benzo(ghi)perylene	-0.140	0.9967	27.6	-0.418	0.9999	61.9

Table 5: Slopes, correlation coefficients and extracted percentages determined from the performed depletion experiments according to Zimmermann et al.[2] for samples containing 19 mL of water and an initial concentration of 50 ng L-1 PAHs for the first extraction by a classic SPME fiber (0.6 µL) and a PAL SPME Arrow (15.3 µL)

Compound	SPME fiber			PAL SPME Arrow		
	Slope	R^2	E (%)	Slope	R^2	E (%)
Naphthalene	-0.023	0.9903	5.2	-0.184	0.9950	33.5
Acenaphthylene	-0.028	0.9842	6.2	-0.254	0.9997	44.3
Acenaphthene	-0.041	0.9946	9.0	-0.326	0.9981	52.8
Fluorene	-0.050	0.9927	10.9	-0.324	0.9979	52.5
Phenanthrene	-0.060	0.9972	12.9	-0.285	0.9947	48.1
Anthracene	-0.071	0.9930	15.1	-0.395	0.9908	59.7
Pyrene	-0.097	0.9956	20.1	-0.317	0.9945	51.8
Fluoroanthene	-0.096	0.9972	19.9	-0.290	0.9991	48.7
1,2-Benzanthracene	-0.137	0.9925	27.1	-0.358	0.9901	56.1
Chrysene	-0.071	0.9235	15.1	-0.278	0.9840	47.3
Benzo(b)fluoroanthene	-0.172	0.9938	32.7	-0.646	0.9940	77.4
Benzo(k)fluoroanthene	-0.176	0.9878	33.4	-1.199	0.9916	93.7
Benzo(a)pyrene	-0.156	0.9958	30.2	-0.689	0.9998	79.6
Indeno(1,2,3 cd)pyrene	-0.159	0.9845	30.7	-0.739	0.9999	81.8
Dibenz(ah)anthracene	-0.142	0.9926	27.8	-1.084	0.9999	91.8
Benzo(ghi)perylene	-0.140	0.9967	27.6	-0.704	0.9999	80.2

Depletion curves of these measurements and their corresponding linear correlations and trend lines are shown in Figures 21 and 22. The slope of the logarithmic depletion curves and their linear correlations are also included in Tables 4 and 5, demonstrating sufficiently good correlations (> 0.98) for all analytes.

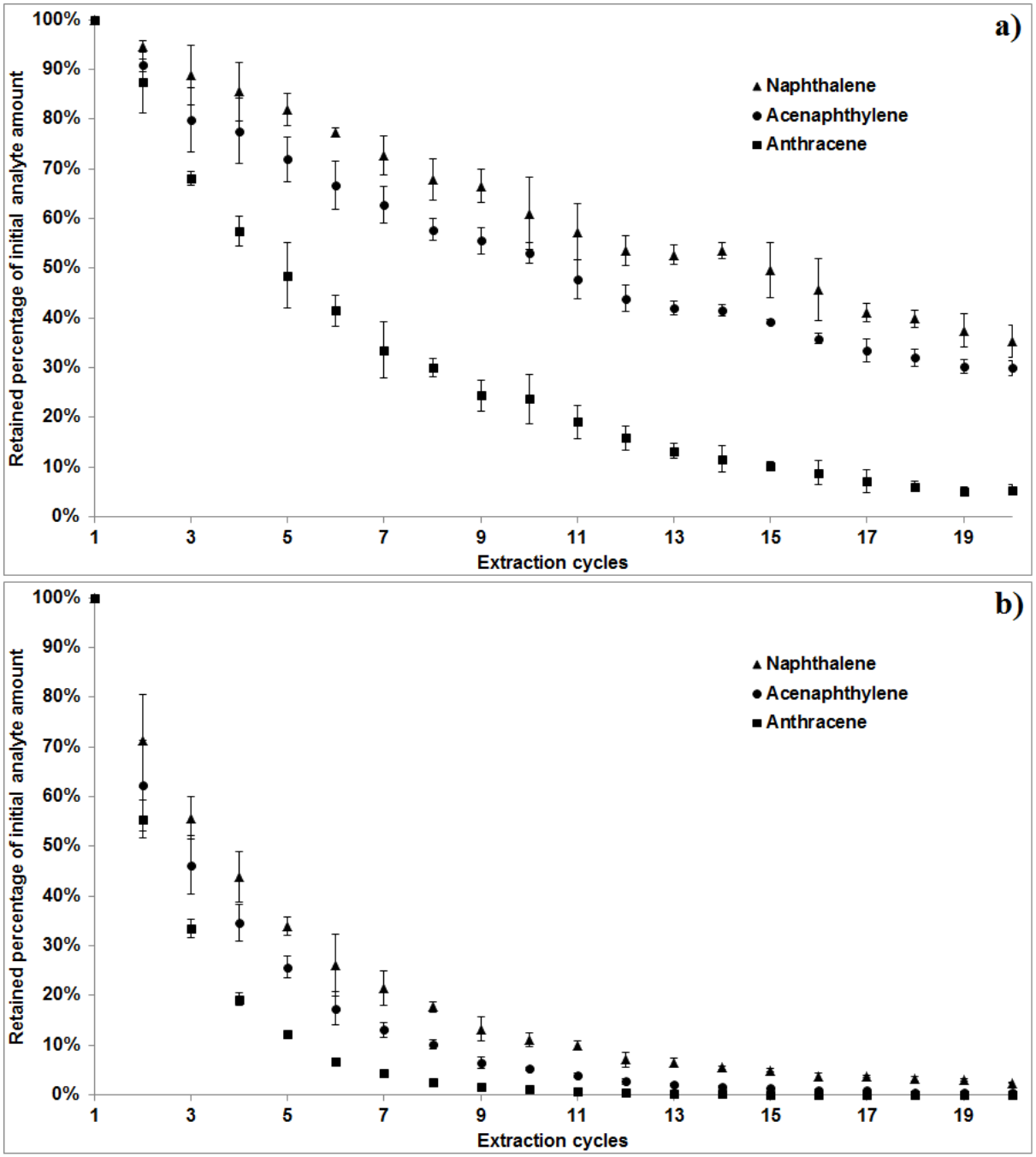

Figure 21: Depletion curves for three exemplary PAHs, extracted by a classical SPME fiber (100 µm x 10 mm, 0.6 µL) (a) and a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL) (b)

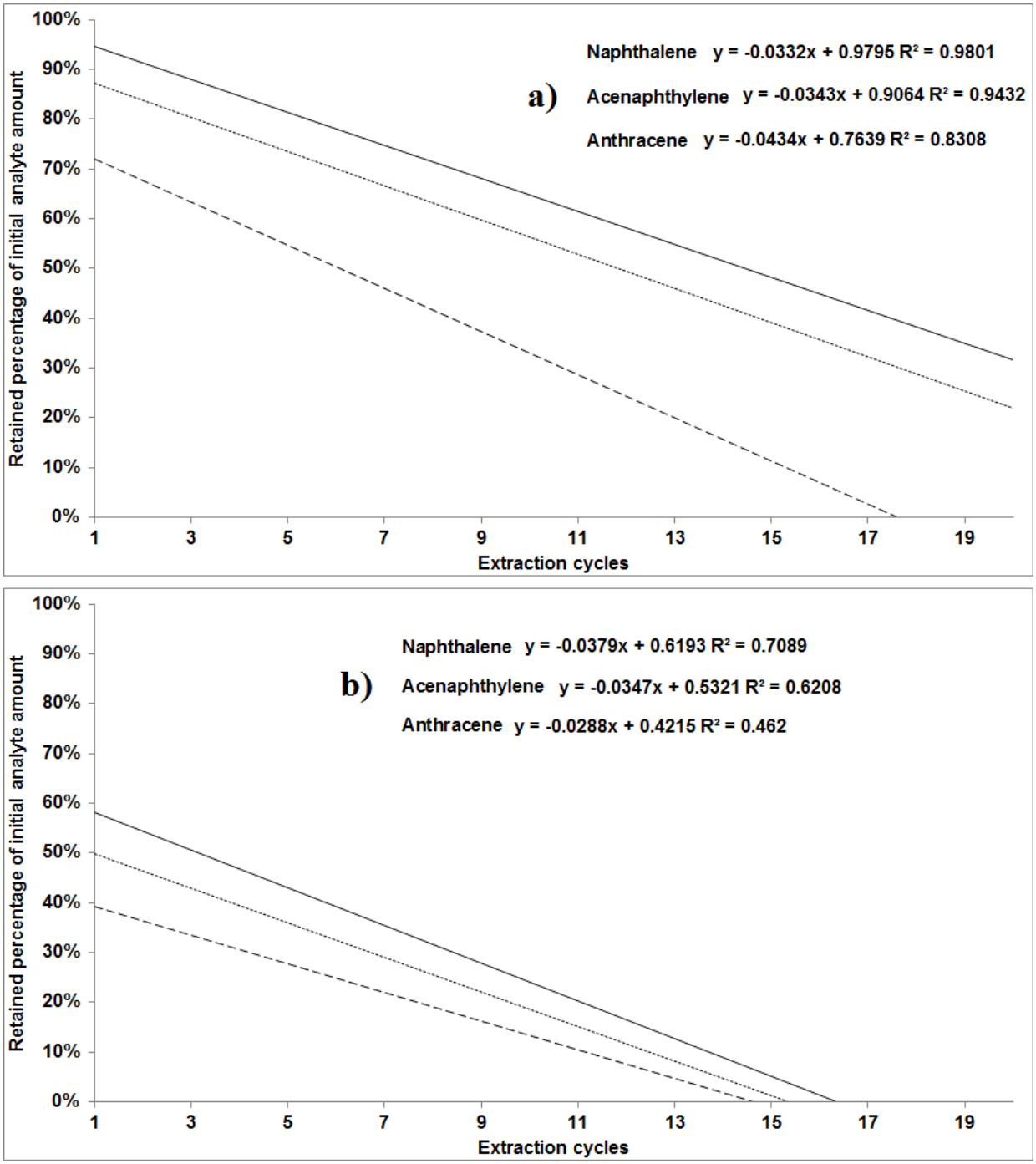

Figure 22: Linear trend lines and correlations for the depletion curves of naphthalene (continuous line), acenaphthylene (dotted line), anthracene (dashed line), extracted by a classical SPME fiber (100 µm x 10 mm, 0.6 µL) (a) and a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL) (b), demonstrating more rapid exhaustion of analytes by PAL SPME Arrow

Extraction efficacy. Further investigation on the extraction behavior of PAL SPME Arrow was conducted by calculating the recoveries that are to be expected theoretically from PDMS-based extraction techniques with different phase volumes. As representative examples we selected a commonly available variant of classical SPME fibers, a PAL SPME Arrow and an SBSE device. Using K_{fs} values from literature[56], we calculated the theoretically extracted percentages for the aforementioned extraction phases and three model analytes under equilibrium conditions as shown in Figure 23.

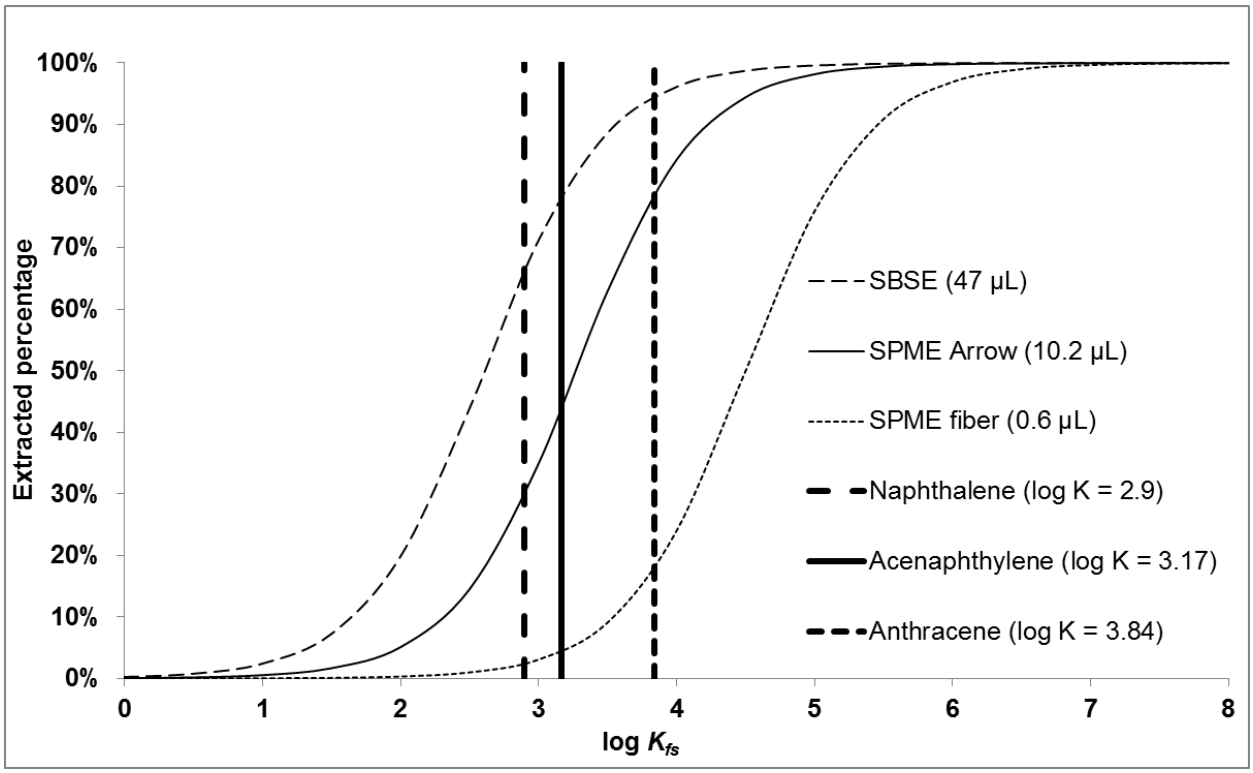

Figure 23: Theoretically extracted percentages for three extraction techniques and exemplary PAHs under equilibrium conditions calculated for an aqueous sample volume of 19 mL with indicated PDMS volumes and log K*fs* values from literature[33]

For an initial estimation of PAL SPME Arrows' extraction capabilities, samples were prepared with identical analyte concentrations of 10 ng L^{-1} each and were extracted either by a classical SPME fiber (100 µm x 10 mm, 0.6 µL), or by a SPME Arrow (100 µm x 20 mm, 3.8 µL). Results are depicted in Figure 24 and show the increase in peak areas in case of the larger sorption phase volume. An approximately 2.5-fold increased extraction yield in case of SPME Arrow can be estimated from the figure.

The sorption kinetics of the two fibers, in terms of required extraction times in order to reach an equilibrium state, are thereby in agreement, since the phase thickness as their main determining factor[18] remains unchanged.

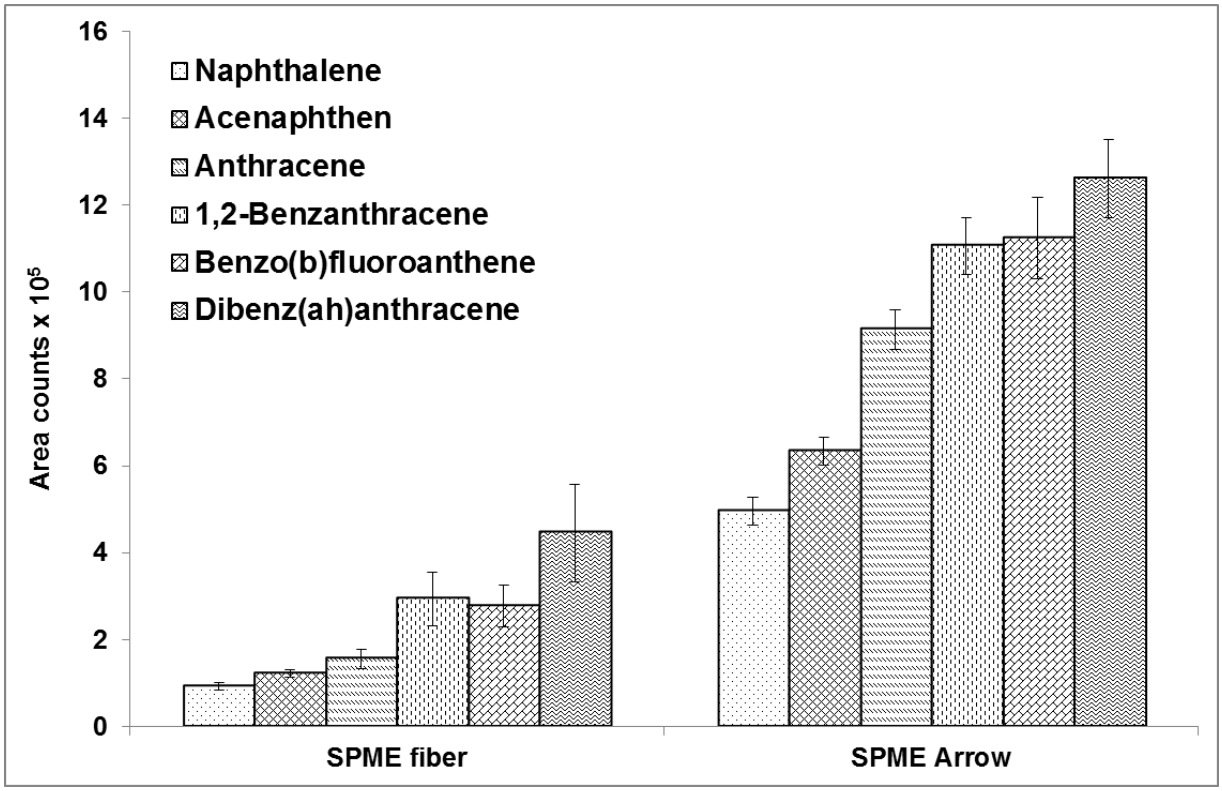

Figure 24: Peak area comparison for aqueous samples containing 10 ng L-1 of PAH each, extracted by a classical SPME fiber (100 µm x 10 mm, 0.6 µL) and a SPME Arrow (100 µm x 20 mm, 3.8 µL) via five-fold replicated immersion extraction by PDMS, shown for six exemplary PAH

Comparison to literature. To enable a statistical comparison of achievable detection limits for PAL SPME Arrow with classical SPME fibers, we determined the method detection limits (MDL) according to Keith et al.[59], as well as relative standard deviations.

Using PAL SPME Arrow (250 µm x 20 mm, 10.2 µL), it was possible to calibrate in concentration ranges as low as 0.5 to 2.5 ng L^{-1} for all 16 EPA PAHs. Results are displayed in Table 6 terms of MDL and RSD values for calibrations performed in ultrapure water and filtrated groundwater. Linear ranges and correlation coefficients for these calibrations can be found in Table 7. An exemplary result of a linear dynamic range test is shown in Figure 25.

Table 6: Calibration results obtained with PAL SPME Arrow (250 µm x 20 mm, 10.2 µL) in ultrapure water and groundwater: MDL values (calculated with a 99% confidence interval) and relative standard deviations (RSD)

Compound	Ultrapure water		Groundwater	
	MDL (ng L^{-1})	RSD (%) (at 10 ng L^{-1})	MDL (ng L^{-1})	RSD (%) (at 10 ng L^{-1})
Naphthalene	0.3	5.7	1.2	6.9
Acenaphthylene	0.2	6.0	0.9	4.8
Acenaphthen	0.1	7.1	2.3	13.0
Fluorene	0.2	5.6	1.9	10.6
Phenanthrene	0.2	5.5	/	/
Anthracene	0.3	7.6	/	/
Pyrene	0.2	6.4	/	/
Fluoroanthene	0.2	6.2	/	/
1,2-Benzanthracene	0.1	6.2	0.7	3.8
Chrysene	0.1	11.0	0.8	4.3
Benzo(b)fluoroanthene	0.2	10.5	0.6	3.4
Benzo(k)fluoroanthene	0.2	8.6	0.6	3.2
Benzo(a)pyrene	0.3	7.2	0.5	2.4
Indeno(1,2,3 cd)pyrene	0.8	9.2	/	/
Dibenz(ah)anthracene	0.6	11.3	0.7	3.8
Benzo(ghi)perylene	0.8	11.9	0.6	3.4

Table 7: Calibration results obtained with a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL) in ultrapure water and groundwater: Linear ranges and correlation coefficients

Compound	Ultrapure water		Groundwater	
	Lin. Range (ng L^{-1})	Correlation coefficient	Lin. Range (ng L^{-1})	Correlation coefficient
Naphthalene	0.3 - 500	0.9878	1.2 - 500	0.9981
Acenaphthylene	0.2 - 500	0.9986	0.9 - 500	0.9995
Acenaphthen	0.1 - 500	0.9817	2.3 - 500	0.9976
Fluorene	0.2 - 500	0.9850	1.9 - 500	0.9861
Phenanthrene	0.2 - 500	0.9976	/	/
Anthracene	0.3 - 500	0.9966	/	/
Pyrene	0.2 - 500	0.9907	/	/
Fluoroanthene	0.2 - 500	0.9951	/	/
1,2-Benzanthracene	0.1 - 500	0.9905	0.7 - 500	0.9943
Chrysene	0.1 - 500	0.9917	0.8 - 500	0.9909
Benzo(b)fluoroanthene	0.2 - 500	0.9965	0.6 - 500	0.9949
Benzo(k)fluoroanthene	0.2 - 500	0.9949	0.6 - 500	0.9982
Benzo(a)pyrene	0.3 - 500	0.9994	0.5 - 500	0.9983
Indeno(1,2,3 cd)pyrene	0.8 - 500	0.9939	/	/
Dibenz(ah)anthracene	0.6 - 500	0.9990	0.7 - 500	0.9997
Benzo(ghi)perylene	0.8 - 500	0.9941	0.6 - 500	0.9998

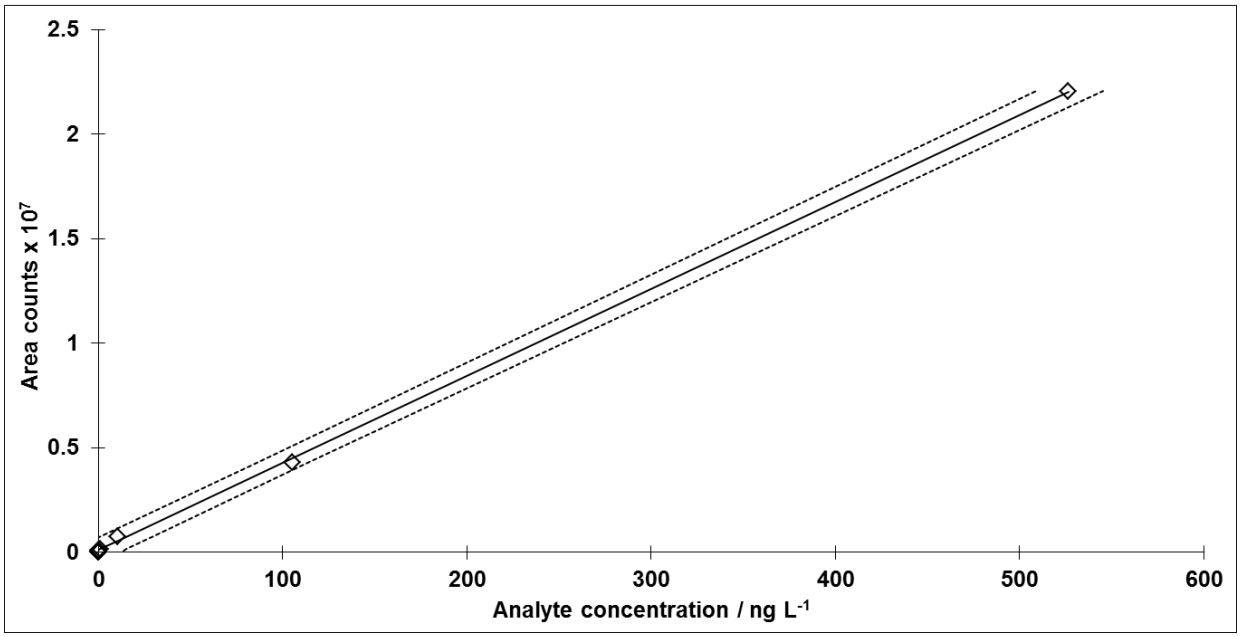

Figure 25: Exemplary regression line for the linear dynamic range test of naphthalene. Correlation coefficient: 0.99989, Linearity test value (F-Test according to Mandel): 1.66 with F: 98.5 (passed)

In accordance to literature[60], it was impossible to determine freely dissolved PAHs via SPME fiber or PAL SPME Arrow in groundwater samples with significant content of particulate organic matter (POM).

After removal of POM (along with sorbed compounds) via filtration, spiking of groundwater samples enabled determination of PAHs from the freely dissolved fraction with the following exceptions due to matrix interference: Phenanthrene, anthracene, pyrene, fluoroanthene and indeno(1,2,3 cd)pyrene.

Table 8 displays LODs and RSDs for PAL SPME Arrow and comparable techniques. While Cheng et al.[49] extrapolated the LOD values for their classical SPME fibers from the standard deviation of their results at the lowest calibration point (10 ng L^{-1}), the results presented herein were calculated from measurements at 0.5 ng L^{-1} for reagent water based samples and at 5 ng L^{-1} for groundwater samples.

Carrera et al.[55] achieved LODs that are similar to the ones generated with PAL SPME Arrow, by extracting a 100 mL water sample for 14 hours with a 500 µm x 20 mm SBSE bar. We calculated the sorption phase volume on these bars to be 47 µL, which would be approx. 3-fold larger as the largest available PAL SPME Arrow phase.

Determined MDLs for PAL SPME Arrow are generally more similar to those generated with the SBSE bars and approximately one order of magnitude better than those of the classical SPME fibers. In contrast to SBSE though, these results have been achieved with a fully automated method. The corresponding RSD values are thereby in the range of 5-12% which is acceptable in such small concentration ranges and in good agreement with literature.

Table 8: MDL and RSD results obtained with PAL SPME Arrow (250 µm x 20 mm, 10.2 µL) for PAHs in water in comparison with literature data for classical SPME fibers and SBSE bars (/ = not determined) (MDL values calculated with a 99% confidence interval)

Compound	PAL SPME Arrow		SPME (Cheng et al.)[49]		SBSE (Carrera et al.)[55]	
	MDL (ng L^{-1})	RSD (%) (at 10 ng L^{-1})	LOD (SD X 3)	RSD (conc. at S/N=3 x 3)	LOD (conc. at S/N=3 x 3)	RSD (%) (at 50 ng L^{-1})
Naphthalene	0.3	5.7	2.7	9.0	/	/
Acenaphthylene	0.2	6.0	1.8	6.0	0.1	/
Acenaphthene	0.1	7.1	0.9	3.0	/	/
Fluorene	0.2	5.6	3	10.0	0.1	8.3
Phenanthrene	0.2	5.5	2.1	7.0	0.1	1.1
Anthracene	0.3	7.6	2.1	7.0	0.2	2.1
Pyrene	0.2	6.4	3.6	12.0	0.2	/
Fluoroanthene	0.2	6.2	2.1	7.0	0.2	/
1,2-Benzanthracene	0.1	6.2	2.1	7.0	0.2	6
Chrysene	0.1	11.0	1.5	5.0	0.2	10.6
Benzo(b)fluoroanthene	0.2	10.5	2.7	9.0	0.1	/
Benzo(k)fluoroanthene	0.2	8.6	1.8	6.0	0.1	/
Benzo[a]pyrene	0.3	7.2	3.6	12.0	0.1	/
Indeno(1,2,3 cd)pyrene	0.8	9.2	3.6	12.0	0.3	/
Dibenz(ah)anthracene	0.6	11.3	/	/	0.3	/
Benzo(ghi)perylene	0.8	11.9	1.8	6.0	0.3	/

Exemplary leaching experiment. For the roofing felt samples, naphthalene and acenaphthylene were the only EPA PAHs that could be measured from the freely dissolved fraction of the sample. This was expected, since the material pieces inside the vials act as a second organic, hydrophobic phase. Since the sorptive properties of PAHs increase with their molecular weight, larger compounds are difficult to remove from this phase without a solvent extraction step. In addition to the two above mentioned PAHs, further compounds have been tentatively identified via their mass spectral information in the NIST (national institute of standards and technology) library. These compounds and their estimated concentrations (converted from 300 mg to 1 g) are summarized in Table 9. Latter concentrations can be expected to be leached into one liter of water, which is exposed to one gram of roofing felt under the extraction conditions given above. Since the used calibration standards contained the 16 EPA PAHs, these results were estimated using the calibration functions of naphthalene (for naphthalene and 2-vinylnaphthalene) and acenaphthylene (for all other compounds). It should however be noted, that only PAH and structurally similar substances such as heterocycles or substituted PAH were taken into account during these measurements.

Table 9: Results for roofing felt extractions with ultrapure water, measured with a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL): Leached concentrations (estimated from calibrations for naphthalene and acenaphthylene for all other compounds) and relative standard deviations (RSD) at calculated concentrations

Compound	CAS-Nr.	Concentration leached into water per gram (ng L^{-1})	RSD (%)
Naphthalene	91-20-30	15	5.2
2-Vinylnaphthalene	827-54-3	27	4.4
Biphenylene	259-79-0	14	9.4
[2-(Naphth-2-yl)vinyl]-methyl sulfone	Not available	13	4.9
1-Isoquinolinecarbonitrile	1198-30-70	24	5.2
5-Isoquinolinecarbonitrile	27655-41-0	13	4.6
Benz(a)azulene	246-02-6	16	5.1
Acenaphthylene	208-96-8	38	5.6
2,3-Naphthalenedicarbonitrile	22856-30-0	96	4.0
Diazene, 1-methoxy-2-[2-(1-naphthyl)ethenyl]-2-oxide-	Not available	14	3.3

Despite the minor concentrations recovered in this small-scale experiment, the large quantities of, e.g., bitumen-based water proofing materials that are applied globally, could still account for a significant contribution to the overall anthropogenic discharge of PAHs into the environment. Further assessment of these contributions should involve influences by temperature, acidity and UV-radiation.

2.4 Conclusions

With PAL SPME Arrow (Figure 26), it was possible to measure PAHs from the freely dissolved fraction in aqueous samples down to the low ng L^{-1}-range or even below. For many compounds this also applied if they had to be extracted from filtered groundwater. Achieved extraction yields and resulting sensitivities clearly benefit from the enlarged sorption phases of PAL SPME Arrow while all advantages of the classical SPME fiber are maintained.

As demonstrated in correlation with SBSE literature, the beneficial effect of increased sorption phase volumes declines with further increasing phase volumes, since the phase ratio between sample and sorption phase becomes less optimal unless significantly larger sample volumes in the range of liters are used. Since the handling of latter sample dimensions as well as the SBSE technique itself is more difficult to automate, PAL SPME Arrow might be a more effective solution in terms of combining maximal extraction efficiency with a fully automatable extraction device and sample size.

Figure 26: PAL SPME Arrow in a stirred water sample

The only drawback of this new option in terms of the mandatory, slight widening of the injector port, is considered less critical when compared to the additional thermal desorption equipment that is required for SBSE bars.

In addition, the increased mechanical robustness of PAL SPME Arrow facilitates extended, unattended measurement series typically found in routine laboratories.

Lastly, the enlarged sorption phase dimensions and the design principle of PAL SPME Arrow can be advantageous for the realization of new sorption phase materials- and combinations. For instance, the enlarged surface area might enhance the effects of carbon nanomaterials, which exhibit promising potential as upcoming sorption phase materials[61,62].

3 Efficiency, reliability and speed in headspace extraction using PAL SPME Arrow

3.1 Introduction

While the previous chapter was focused on immersive sampling with PAL SPME Arrow, it was also determined how suitable the device is for headspace extraction. Headspace SPME has the general advantage of a faster extraction of analytes compared to immersion extraction since the diffusion boundary layer is smaller in this case[9,10] This has for example been used for sampling of volatile organic contaminants such as benzene, toluene, ethylbenzene and xylenes (BTEX) from aqueous samples[11]. Furthermore, the sample headspace functions not only as a transmission medium but also as a barrier that prevents contamination of the sorption phase by matrix components of the sample[63,12].

The latter aspect is of special advantage during the extraction of sample matrices with potentially contaminative properties for the fiber such as milk, which has for example been examined for PAHs[64]. In another example for challenging sample matrices, beer was investigated for alcohols and esters via HS-SPME and static headspace analysis via loop. Ultimately, HS-SPME provided lower limits of detection in this case, while both methods exhibited good repeatability[65].

In the second chapter of this thesis, PAL SPME Arrow has already demonstrated increased method sensitivity, compared to the classical SPME fiber, for the immersion extraction of PAH from various aqueous samples[7]. The aim of the work in this chapter was to elucidate, whether the same benefit occurs in case of headspace extractions. Furthermore, it was evaluated, if the enlarged sorption phase volume of the device can also be used in order to minimize the required sampling time without significant losses in method sensitivity. In addition, different sorption phase dimensions of PAL SPME Arrow were evaluated here.

For a thorough comparison between the classical SPME fiber and PAL SPME Arrow, only one sorption phase dimension (250 µm of thickness and 20 mm of length for PAL SPME Arrow), and just one type (PDMS) were used in combination with only one class of analytes (PAHs) for the previous chapter. Here, different dimensions and types were also evaluated alongside different classes of analytes in order to achieve a broader basis for comparison.

3.2 Experimental

Aqueous samples were prepared similar to the previous chapter for all analyte classes, with the only exception of a liquid sample volume of 10 mL. While the other measuring parameters remained as in the previous chapter, the preconditioning and extraction temperature was changed to 60°C. This facilitates the transition of the analytes into the sample headspace during the 15 min of fiber exposure, which were always used in this chapter unless stated otherwise for individual experiments. These starting values were chosen on the basis of personal communication with professionals. Required extraction times for headspace sampling with PAL SPME Arrow were evaluated in detail later on as will be shown at the end of this chapter.

All other conditions for injection, chromatographic separation, mass spectrometric detection and method automation were identical to the conditions described in the last chapter. Additionally used PAL SPME Arrows were: 100 µm X 20 mm with 3.8 µL PDMS and 100 µm X 20 mm with 3.8 µL Carbo WR (wide range).

3.3 Results and discussion

Method sensitivity comparison. For an initial estimation of PAL SPME Arrow's effect when used for HS-SPME methods, a comparison to classical SPME fibers was performed with regard to achievable sensitivities. As basis for this comparison, resulting mean peak areas for the individual target analytes were used. Measurement conditions were identical except for the used

fibers. Measurement series were carried out using aqueous PAH samples with an analyte concentration of 25 ng L^{-1} that were measured as five-fold replicates with either a classical SPME fiber or two PAL SPME Arrow PDMS sorption phases with different volumes (100 µm x 10 mm, 0.6 µL, 100 µm x 20 mm, 3.8 µL and 250 µm x 20 mm, 10.2 µL). A graphical comparison of the obtained mean peak areas in these experiments is shown in Figure 27. Results indicate that the enlarged sorption phases of PAL SPME Arrow have significant effect on method sensitivity.

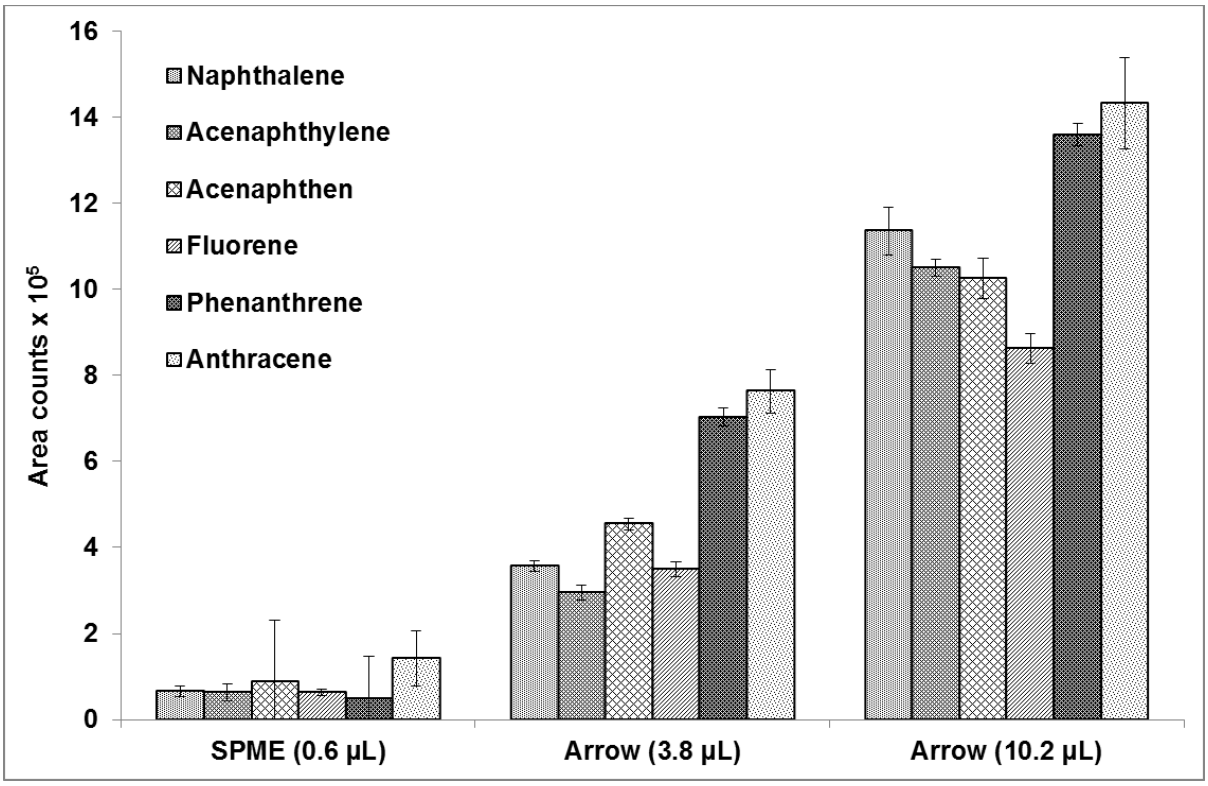

Figure 27: Mean peak areas and RSDs (error bars) obtained for HS-SPME analysis of volatile PAHs using a classical SPME fiber as well as two PAL SPME Arrow PDMS sorption phases with different volumes (100 µm x 20 mm, 3.8 µL and 250 µm x 20 mm, 10.2 µL).

Following this initial comparison of sensitivity, calibrations in the range of 5 to 50 ng L^{-1} were performed in order to evaluate if concentrations of volatile PAHs can be determined in the lower ng L^{-1} concentration range via headspace extraction by PAL SPME Arrow.

Results are displayed in Figures 28 to 30 as well as Table 10 and indicate suitability of the method especially for the four most volatile PAHs up to fluorene (linear correlation coefficient > 0.99). Phenanthrene and anthracene could also be measured with this method, however with less than optimal linearity (linear correlation coefficients of 0.959 and 0.9884 respectively). MDLs and RSDs were also determined, are displayed in Table 10 and should be considered as tentative for phenanthrene and anthracene due to insufficient linearity. These results can be compared to literature using headspace extraction via SBSE (HS-SBSE) as a reference. Latter technique is also a static enrichment solution with an increased sorption phase volume compared to the classical SPME fiber. For instance, Grossi et al. found linear correlations between 0.9602 and 1.0, measurement repeatability between 2.1 and 14.8 % and MDLs as low as 10 ng L^{-1} for the headspace extraction of pesticides in a similar concentration range via HS-SBSE. These results correlate well to the ones presented here, especially in terms of linearity and repeatability.

Prieto et al. reported MDLs between 0.4 and 5 ng L^{-1} for HS-SBSE extractions of methylmercury and butyltin species from aqueous samples[66], which is also in good correlation to the MDLs shown in this study. For a straightforward comparison of the results with values that can be obtained using classical SPME fibers, data presented by Campo et al. are included in Table 10[67]. While this paper contained only limits of quantification (LOQ), which are inherently higher than MDLs, the paper contained no information on how these values were determined. Assuming that, e.g., the S/N-based method was used that usually yields lower LOQs and LODs than the MDL method used here[59], values appear well comparable. Reported RSDs are similar or higher than the ones presented in this work while the MDL (LOQ) are roughly higher by a factor of 10 for most analytes.

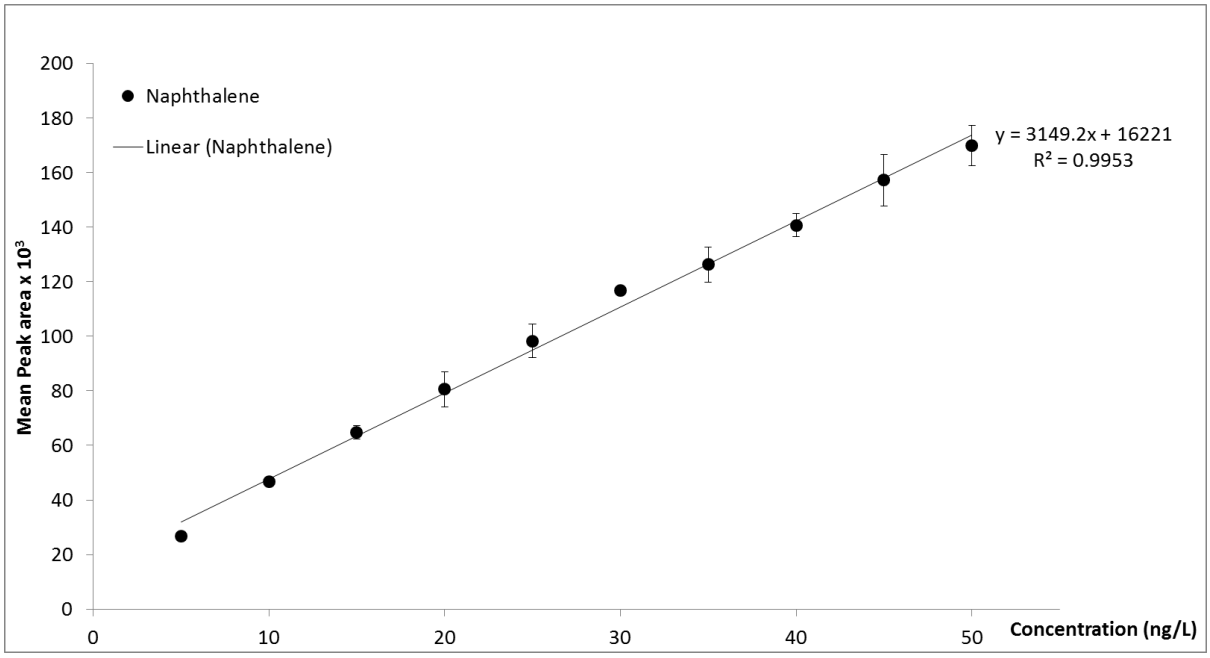

Figure 28: Calibration curve of naphthalene extracted from the headspace of aqueous samples with a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL)

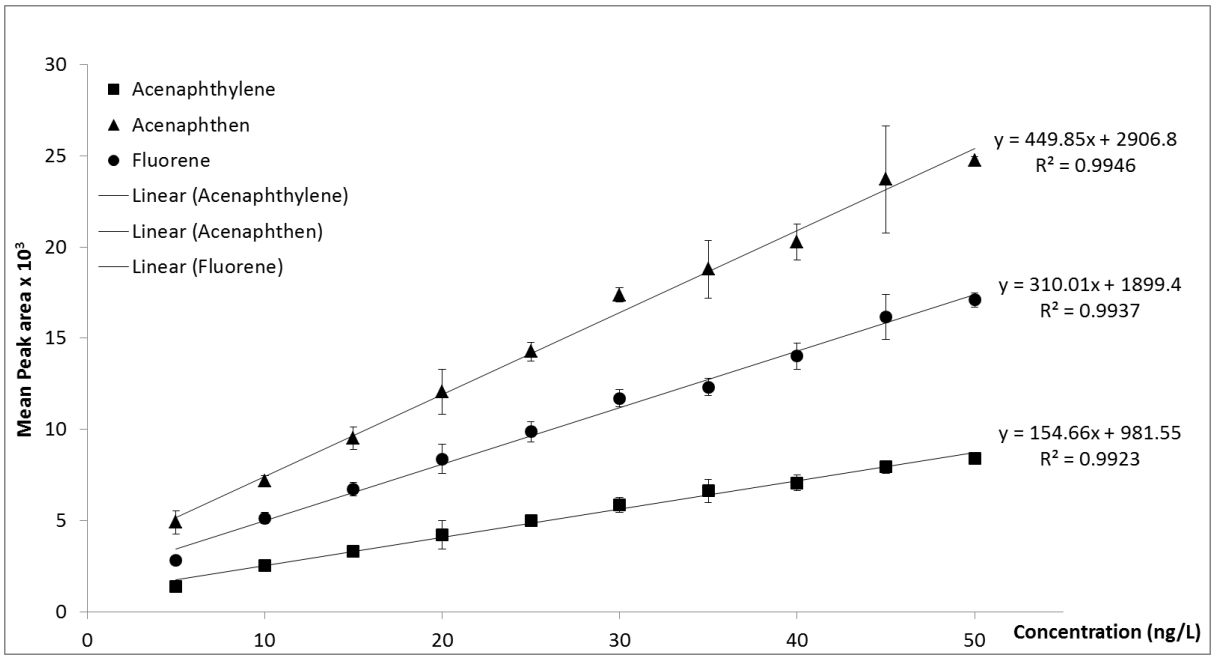

Figure 29: Calibration curves of acenaphthylene, acenaphthen and fluorene extracted from the headspace of aqueous samples with a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL)

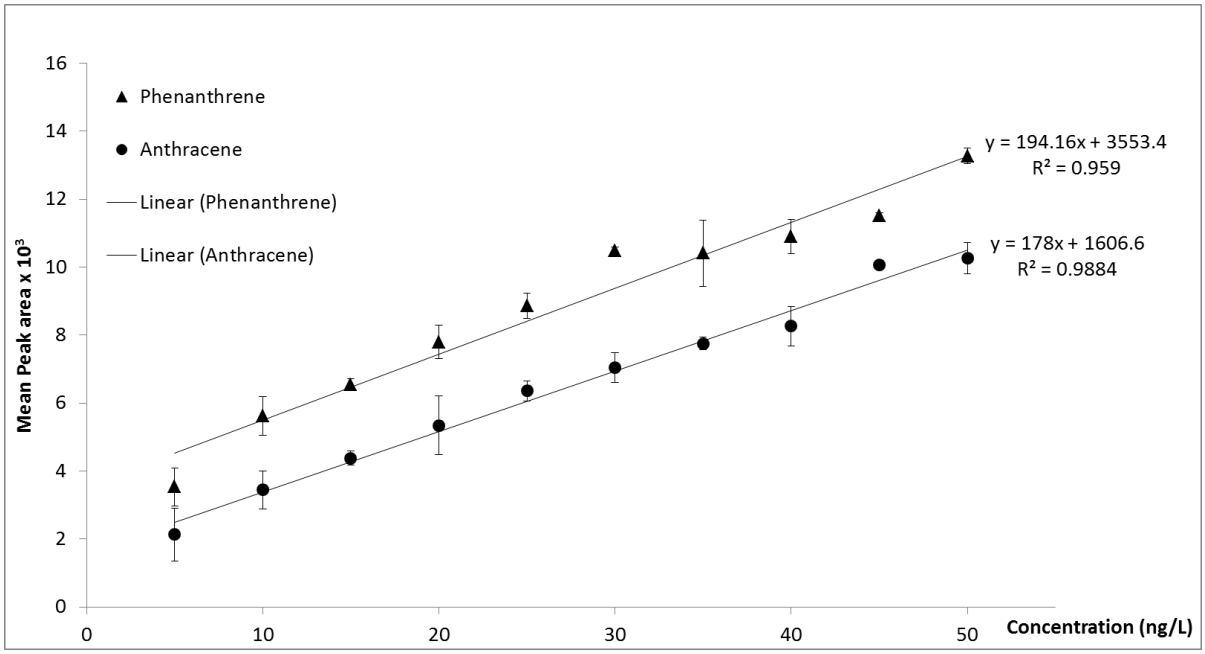

Figure 30: Calibration curve of phenanthrene and anthracene extracted from the headspace of aqueous samples with a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL)

Table 10: RSDs and MDLs for the PAH extracted from the headspace of aqueous samples with a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL). RSD and MDL were calculated at 10 ng L^{-1}

Parameter	Naphthalene	Acenaphthylene	Acenaphthen	Fluorene	Phenanthrene	Anthracene
RSD (%)	1.8	5.8	2.7	1.3	2.4	2.6
MDL (ng L^{-1})	0.6	4.0	0.6	0.3	1.0	0.7
LOQ (ng L^{-1}) [67]	22.8	4.1	6.0	4.6	5.1	2.3
RSD (%) [67]	19	14	22	36	8	16

BTEX extraction by Carbo WR. In another series of experiments, measurements were carried out for another combination of analytes and sorption phase material. Figure 31 and Table 11 show corresponding results for BTEX, which were extracted not as in the previous measurements with PDMS sorption phases but with a novel Carbo WR-named combination of PDMS with highly porous carbon particles. This new phase demonstrated very good repeatability at the cost of a slightly reduced sensitivity.

In comparison to literature, however, the obtained values are still superior to results that could, e.g., be generated using classical SPME fibers that were coated with a novel sorption phase blend containing carbon nanotubes. In the latter example, MDLs were in the range of 10 to 40 ng L^{-1}, while RSDs were in a range of 5.9 to 8.1%[68]. In another example, a classical SPME fiber with a PDMS/DVB coating was used for sampling of BTEX from groundwater. In this work Gebara et al. found MDLs between 30 and 50 ng L^{-1}[69].

Again, sensitivities of classical SPME fibers and PAL SPME Arrows with similar sorption phase compositions differ by a factor of approx. 10 in favor of the PAL SPME Arrow. This corroborates the other results presented in chapter 2. However, the immersion-based extraction used in that chapter resulted in MDLs that were generally lower by another factor of 10 compared with HS extraction.

The effect of different sorption phase materials on the achievable detection limits was limited in case of this experiment (compare Tables 10 and 11). This observation will be subject to closer investigation in the next chapter of this work for a broader range of analytes.

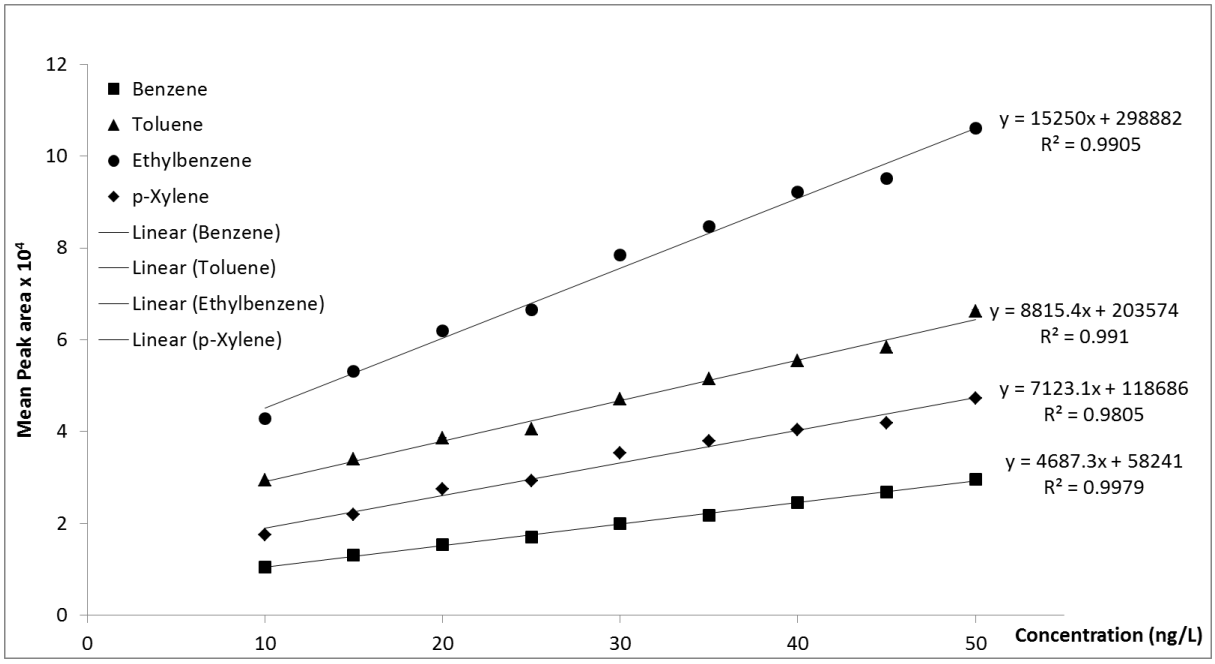

Figure 31: Calibration curve of BTEX compounds extracted from the headspace of aqueous samples with a PAL SPME Arrow (Carbo WR 100 µm x 20 mm, 3.8 µL)

Table 11: RSDs and MDLs for BTEX compounds extracted from the headspace of aqueous samples with a PAL SPME Arrow (Carbo WR 100 µm x 20 mm, 3.8 µL); RSD and MDL were calculated at 10 ng L^{-1}

(ng/L)	Benzene	Toluene	Ethylbenzene	p-Xylene
RSD (%)	2.2	2.0	1.6	1.3
MDL	1.7	3.5	3.6	5.1

Pre-equilibrium sampling. Sample preparation times should not govern overall analysis time. Therefore, it was studied if extraction by PAL SPME Arrow may also enable an increased time-efficiency compared to classical SPME fibers due to the higher sensitivity that it provides. In contrast to other measurement series presented in this work, focus here was not on a maximization of method sensitivity and repeatability. Instead the aim was an implementation of PAL SPME Arrow with a saving of time in mind.

This possibility was evaluated by headspace sampling of analytes with high volatility in narrow time intervals. After such short timeframes, the three-phase system (sample solution, headspace, sorbent) is not yet entirely equilibrated but this process is interrupted prematurely.

Thus, only a pre-equilibrium is established[70], and depending on the point of time when this interruption is carried out, additional enrichment time would have resulted in a significant further increase in extraction yield[71,72]. It is therefore important that the extraction time is precisely controlled by the autosampler in order to achieve results with amenable repeatability. This effect is increasingly pronounced with shorter enrichment times and becomes less important with longer enrichment times due to the exponential nature of the extraction process. If an extraction phase is already largely equilibrated after, e.g., 20 min, it makes little difference if it is aborted after these 20 or 21 min.

The results exemplarily depicted in Figures 32 and 33 show a significantly superior enrichment of the target compounds by PAL SPME Arrow in comparison to classical SPME fibers in the same time periods. Enrichment times below 1 min may, however, result in unsatisfactory measurement repeatability, as is especially apparent in Tables 12 and 13, showing detailed RSDs for the other compounds that were used in this study. According to these results, analyses with PAL SPME Arrow are more dependent on sufficiently long extraction times (>1 min) compared to classical SPME fibers.

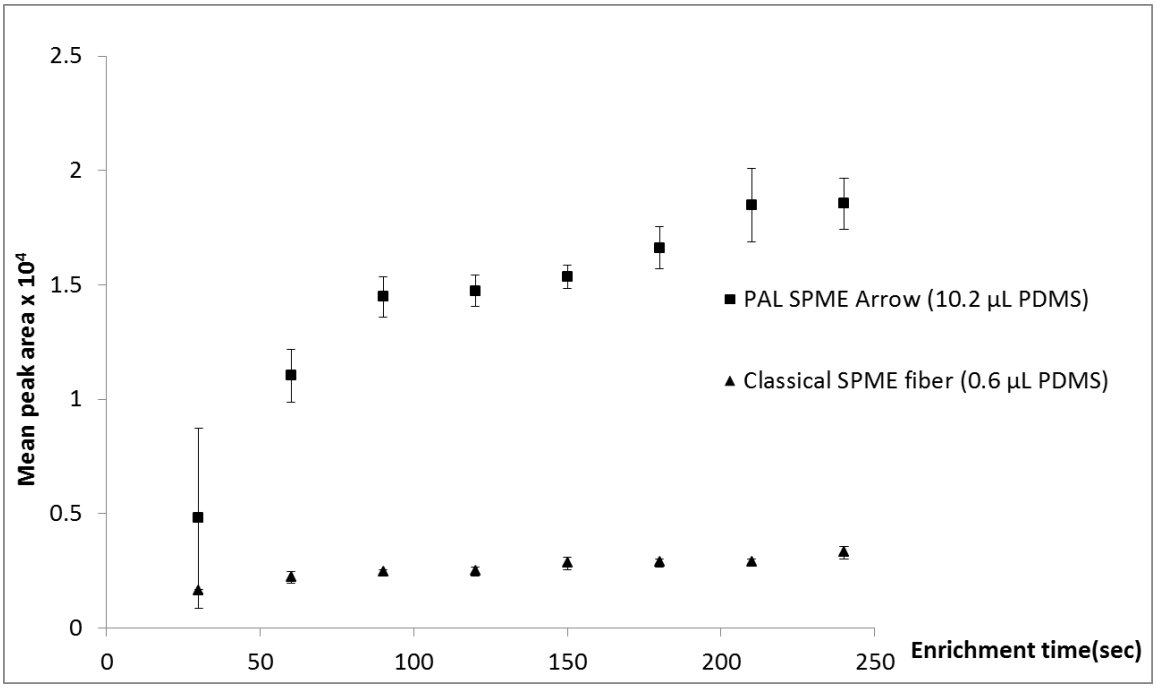

Figure 32: Short-interval enrichment time evaluation for tert-butylbenzene extracted from aqueous samples with a concentration of 1 µg L^{-1}, using a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL) and a classical SPME fiber (100 µm x 10 mm x 0.6 µL)

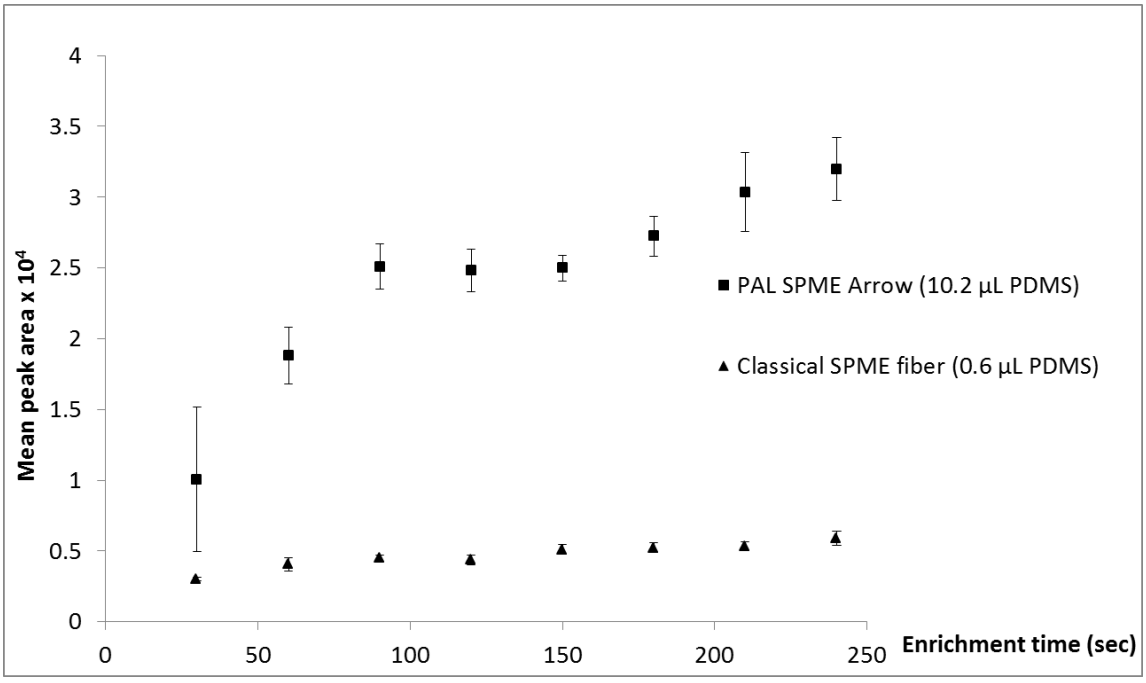

Figure 33: Short-interval enrichment time evaluation for 2-indanol extracted from aqueous samples with a concentration of 1 µg L^{-1}, using a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL) and a classical SPME fiber (100 µm x 10 mm x 0.6 µL)

Table 12: RSDs for volatile compounds extracted from the headspace of aqueous samples with a classical SPME fiber (0.6 µL PDMS) and various short extraction times. RSDs were calculated at 10 ng L^{-1}

Classical SPME fiber RSDs (%)	Enrichment time (sec)							
Compound Names	30	60	90	120	150	180	210	240
Trichloroethylene	4	5	6	7	4	5	6	6
1,2-Dichloropropane	3	6	5	5	6	8	8	9
2,3-Dichloro-1-propene	91	18	5	5	9	7	8	12
Toluene	7	7	4	8	7	5	6	8
Tetrachloroethylene	6	7	7	7	3	5	3	16
1,1,2-Trichloroethane	8	6	4	7	4	4	8	7
Dibromochloromethane	4	4	7	3	4	4	6	8
Ethylbenzene	33	9	5	8	7	6	5	9
1,3-Dimethylbenzene	9	9	5	7	7	6	5	8
o-Xylene	10	8	4	7	6	6	5	8
Styrene	8	7	4	7	6	5	5	7
1-Methylethylbenzene	6	11	5	7	7	7	5	8
N-Benzyl-2-phenethylamine	5	11	5	7	6	7	5	8
1-Chloro-3-methylbenzene	4	9	5	7	5	5	5	7
tert-Butylbenzene	4	11	5	8	10	6	5	8
1,3,5-Trimethylbenzene	82	9	4	7	5	5	5	7
2-Indanol	4	12	5	8	7	7	5	9
1-Methyl-2-ethylbenzene	5	11	5	8	6	6	5	9
1,3-Dichlorobenzene	4	8	5	7	4	5	5	7
1,4-Dichlorobenzene	4	7	4	7	3	4	5	7
Dodecane	49	16	3	5	6	4	5	8
Butylbenzene	5	11	5	7	5	6	5	9
1,2-Dichlorobenzene	4	6	4	6	3	4	4	7
1,3,5-Trichlorobenzene	6	7	4	7	7	4	5	5
1-Methylene-1H-indene	7	3	5	4	4	4	5	4
1,2,3-Trichlorobenzene	6	6	4	6	5	4	5	4

Table 13: RSDs for volatile compounds extracted from the headspace of aqueous samples with a PAL SPME Arrow (250 µm x 20 mm, 10.2 µL) and various short extraction times. RSDs were calculated at 10 ng L^{-1}

PAL SPME Arrow fiber RSDs (%)	Enrichment time (sec)							
Compound Names	30	60	90	120	150	180	210	240
Trichloroethylene	10	11	6	5	3	6	9	8
1,2-Dichloropropane	11	5	3	4	3	3	6	5
2,3-Dichloro-1-propene	8	7	3	4	3	5	6	6
Toluene	6	9	5	5	2	5	8	6
Tetrachloroethylene	6	10	6	5	3	6	9	7
1,1,2-Trichloroethane	8	1	1	3	3	3	4	4
Dibromochloromethane	15	1	1	3	3	2	5	4
Ethylbenzene	8	9	6	5	3	5	9	6
1,3-Dimethylbenzene	8	10	6	5	3	5	9	6
o-Xylene	7	8	5	5	2	4	8	6
Styrene	6	7	4	5	2	4	8	6
1-Methylethylbenzene	9	11	6	6	3	5	9	7
N-Benzyl-2-phenethylamine	8	11	6	5	3	5	9	7
1-Chloro-3-methylbenzene	8	8	5	5	2	4	8	7
tert-Butylbenzene	82	10	6	5	3	6	9	6
1,3,5-Trimethylbenzene	107	83	7	5	2	5	7	8
2-Indanol	51	11	6	6	4	5	9	7
1-Methyl-2-ethylbenzene	7	11	7	6	3	6	9	8
1,3-Dichlorobenzene	8	7	5	5	1	4	8	7
1,4-Dichlorobenzene	8	6	5	5	1	4	8	7
Dodecane	166	111	4	11	4	5	7	13
Butylbenzene	6	10	7	5	4	6	8	8
1,2-Dichlorobenzene	7	5	4	5	1	3	7	7
1,3,5-Trichlorobenzene	8	6	5	5	2	5	8	9
1-Methylene-1H-indene	8	2	3	5	3	1	6	6
1,2,3-Trichlorobenzene	8	4	4	4	1	3	7	8

3.4 Conclusions

Besides its potential for immersion extraction, which was extensively demonstrated in chapter 2, PAL SPME Arrow is also a good option for HS-SPME sampling. It achieves a significant gain in method sensitivity while its repeatability is at least on par with the classical SPME fiber. This conclusion appears to be independent of varying analytes and extraction phase materials as could be demonstrated for two different corresponding combinations. It is furthermore possible to save overall measurement time without sacrificing sensitivity when combining PAL SPME Arrow with short enrichment times. Latter times should however be longer than approx. 60 seconds depending on the target compounds in order to ensure a sufficient equilibration of the thicker fibers with the result of satisfactory measurement repeatability.

4 Systematic Comparison of Static and Dynamic Headspace Sampling Techniques for Gas Chromatography

This chapter has been submitted in a modified form for publication in Analytical Bioanalytical Chemistry within the year 2016

4.1 Introduction

Gas chromatographic (GC) analysis of substances from the headspace of a sample is an efficient technique for avoiding problems associated with liquid sample handling and injection. The inherent matrix separation of headspace sampling reduces the possibility of contaminating, e.g., SPME fibers or the GC injector[64,9].

The basis of this sampling technique is that analytes in an aqueous or another liquid matrix equilibrate with regard to their concentrations between the sample solution and the gaseous phase above. Thereby latter concentrations depend on the temperature, the phase volume ratio and corresponding distribution constants (e.g. for air/water partitioning: K_{aw}) of the individual analytes[9].

When the phase equilibrium is established, the most straightforward approach to headspace analysis is transferring an aliquot of the equilibrated headspace gas to the GC injector via a gas-tight syringe. In this case, sampling is carried out under static conditions inside the sample vessel and is therefore described as static headspace sampling[9].

This indirect sampling provides quick and easy separation of volatile analytes from their matrices, requires negligible additional instrumentation (in its syringe-based, static variant) and is effortless to automate.

By replacing the syringe with a solid-phase microextraction (SPME) device, it is possible to not only abstract a portion of the headspace gas, but to enrich the desired analytes onto a typically polymeric sorption phase[12].

This static enrichment approach combines the inherent matrix separation of the headspace technique with a concentration step and enables higher sensitivity compared to static sampling.

Headspace sampling can as well be carried out in a non-static, dynamic approach. In this case, the sample headspace is depleted of analytes in a continuous or intervallic manner by, e.g., being cycled through a packed sorbent material. Since the headspace is thereby depleted of analytes, a further mass transfer of the latter from the sample solution into the headspace is taking place. As a final result in an idealized scenario, both sample solution and headspace are depleted of analytes, which have been exhaustively transferred to the sorption phase.

Thermal desorption of these analytes can then be carried out in or into the GC injector, resulting in a larger transferred amount of the target compounds than in case of static enrichment techniques (potentially exhaustive transfer) and thus also a further improved method sensitivity[15,9,73,74]. Dynamic headspace extraction in this sense is defined by the fundamental mode of action and goes beyond the more common definition that is typically limited to continuous flow-through (stripping) designs based on a constant stream of gas purging sample solution or headspace. Thus, all methods that rely on a stream of gas for depleting the samples of volatile analytes will be described as dynamic headspace extraction in the following.

The resulting classes, by which headspace sampling techniques will be differentiated in this work are therefore: Static sampling (static sampling by syringe or loop), static enrichment (e.g. SPME) and dynamic enrichment (e.g. ITEX).

The nowadays widespread acceptance of the headspace technique has spawned a multitude of different instrumental approaches. Each of these has its own, distinct advantages and limitations and is most effective in a specific concentration range. Despite this, systematic comparisons of different, available headspace sampling approaches are hardly available.

Studies carried out so far are typically limited to the comparison of two different methods or compare a novel technique to established ones whereby the latter are only represented by literature data[11,75-77,16].

With the aim of realizing such a systematic comparison of headspace sampling methods, individual techniques were separated into the above mentioned, representative classes with respect to their mode of action:

The first set of methods contains the static headspace sampling techniques. This type of sampling can either be performed via the already mentioned syringe, or in a more complex "loop headspace" approach using a setup of pressurized, silanized and temperature-controlled steel capillaries and valves (Figure 34).

An aliquot of the sample headspace is thereby intermediately stored in a loop prior to its delivery to the injector. While this technique is more complex, it offers permanent control of the headspace aliquot's pressure.

In some cases, depending on the concentration and volatility of the target substances, their amounts in the equilibrated sample headspace may be too small for a reliable analysis by the previously described static sampling techniques.

In such cases, enrichment techniques are employed that involve a concentration step for the analytes resulting in improved method sensitivities.

Static enrichment, can be conducted from the sample headspace, e.g., by SPME and PAL SPME Arrow. Despite the fact that this type of extraction is also possible from the sample solution by immersion of the fiber, it is generally preferable to do so from the headspace for sufficiently volatile analytes. The reasons for this are reduced risk of fiber contamination and faster enrichment of analytes [12,14] due to a diminished thickness of the diffusion boundary layer [64,12,14].

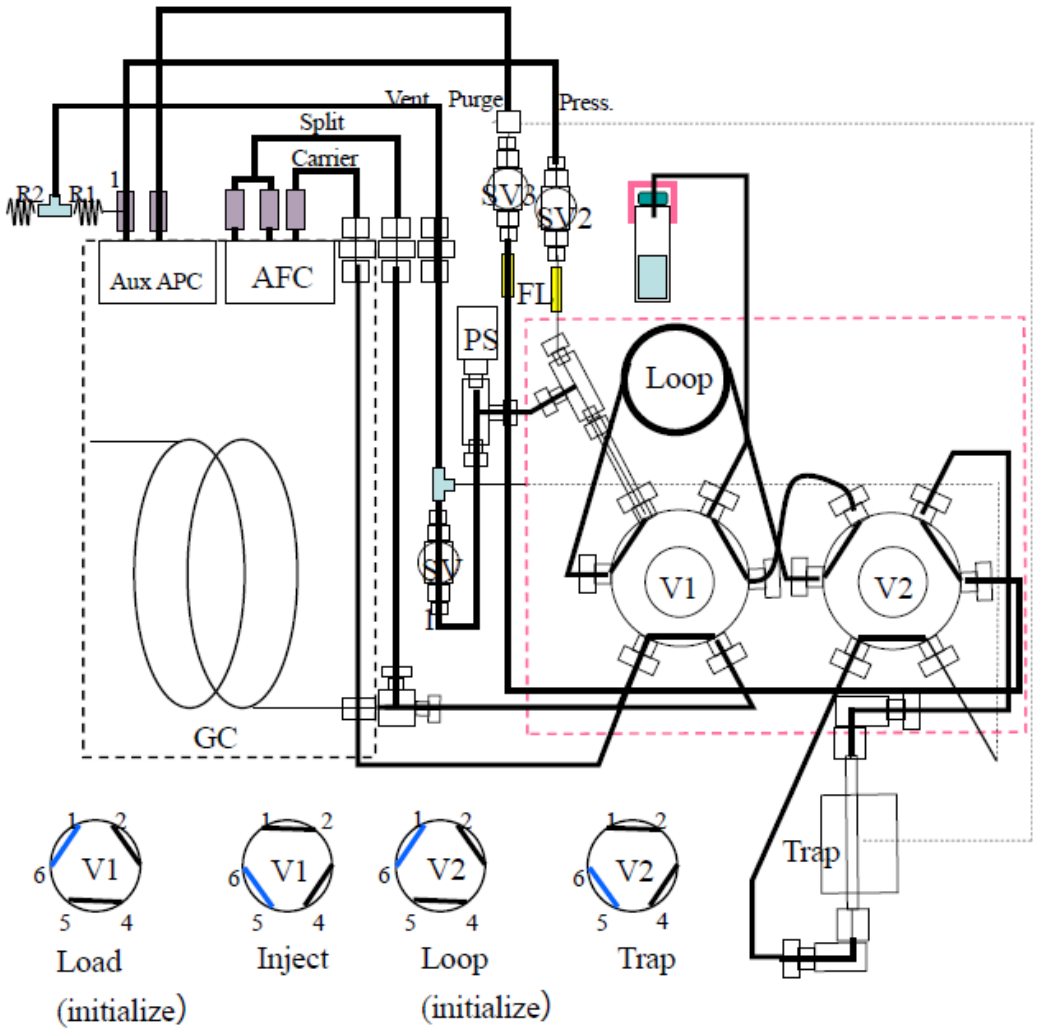

Figure 34: Schematics of the Shimadzu HS-20 automated headspace sampler with V being a six-port valve, SV being a solenoid valve, PS being a pressure sensor, F being a filter, R being a restrictor, APC being an advanced pressure control module and AFC being an advanced flow regulation module. Different valve positions are depicted for loop and trap mode. Reprinted with permission of Shimadzu corporation.

While the possibility of headspace-SPME extraction is known since 1993 [12], and has gained widespread acceptance nowadays[14], the recently introduced PAL SPME Arrow augments this proven concept with enlarged sorption phase volumes and improved mechanical reliability [7]. The enlarged dimensions of its sorption phases in comparison to traditional SPME fibers enable improved sensitivities in combination with a significant increase in mechanical reliability.

In addition, classical SPME fibers and PAL SPME Arrows are both automatable with PAL-type samplers.

Dynamic enrichment, which in contrast to the static enrichment also involves an active cycling of the headspace gas, can be achieved in different ways:

The in-tube extraction (ITEX) system was first published in 2008[13] and cycles the headspace gas multiple times through a sorbent bed in a bidirectional manner. The sorbent is thereby packed into a stainless steel capillary, which tapers into a sideport syringe on one end. The other end is connected to a 1.3-mL headspace syringe which aspirates and dispenses the sample headspace through the sorption phase. For subsequent thermal desorption and the following purge step similar to headspace syringes, the stainless steel capillary containing the sorbent is thereby encased in a heating unit[8].

In its most recent variant ITEX DHS for the PAL RTC sampling platform, an active cooling via a fan was additionally included into the system for a faster cooling of the sorption phase after desorption and purging, resulting in a faster overall cycle time[78]. In Figure 35 it is schematically depicted alongside the other three techniques which are suitable for headspace analysis technique and automation with a PAL sampler.

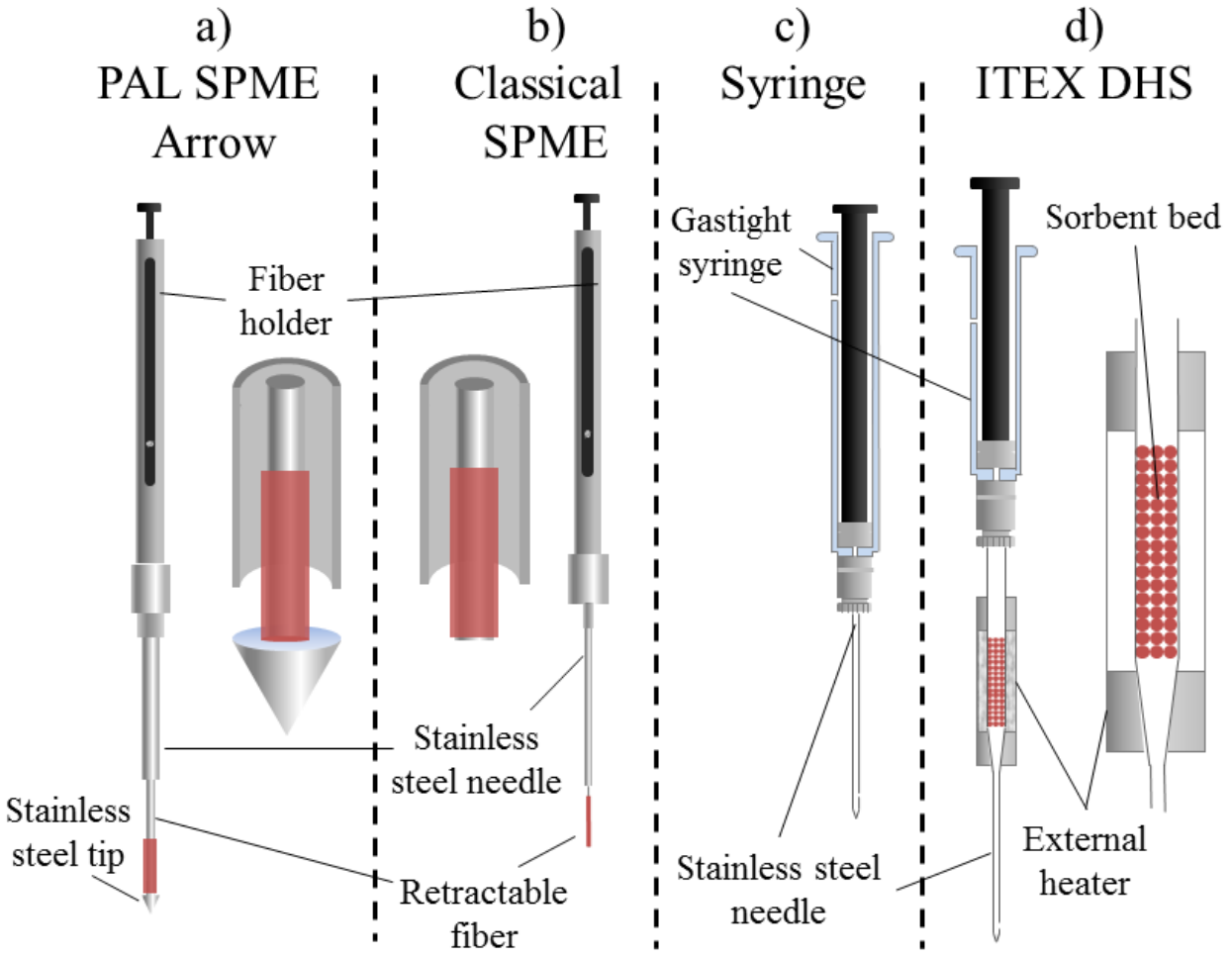

Figure 35: Schematics of the four headspace analysis techniques that were automated with a PAL RTC sampler in this study: PAL SPME Arrow, classical SPME fiber, syringe, ITEX DHS. For the three enrichment techniques the corresponding extraction phases are highlighted in red

Advantages of ITEX DHS include effortless integration into PAL-type autosamplers and straightforward thermodesorption by heating the stainless steel capillary while cycling, e.g., helium from the GC injector via the connected headspace syringe. As a result, no additional, dedicated thermodesorption extension for the GC injector is required for this technique.

The stainless steel capillaries packed with the sorption phases are also easily exchanged, since they are attached to the headspace syringe via a gas tight screw connection. Possible contaminations from “dirty” or foaming samples are therefore quickly remediated.

The used sorbent can furthermore be adapted to the current target compounds, making this technique highly flexible. This is a major advantage over classical purge & trap systems[8].

In another approach to dynamic enrichment, the sample headspace is flushed through a sorbent bed in a unidirectional manner. Sorption phase volumes in these instruments are typically larger than in case of the ITEX technique, since any breakthrough in terms of non-retained analytes is irreversibly lost. The purging of the sample vial can either be carried out in a continuous manner by a stream of gas running through the sample solution, as it is typical for purge & trap solutions, or in intervals that are interrupted by re-equilibrations of the headspace pressure inside the vial. The latter possibility can be combined into a single instrument (such as the Shimadzu HS-20 used in this study) with the previously described loop technique, offering additional sensitivity whenever needed (Figure 34).

The multitude of available techniques makes the informed selection of a particular technique very difficult for a user. This is further pronounced by the fact that in most publications the use of just one technique is described and proposed for further work.

A critical comparison including a thorough evaluation of specific characteristics of each method is lacking so far. In particular, direct comparisons between dedicated headspace-sampling instruments and PAL-type sampler based techniques have not been carried out to date. The aim of this work was therefore to close this gap by providing a comprehensive comparison of the characteristics of six different, commonly available headspace-techniques. To this end, a systematic evaluation of these methods was carried out. For ensuring comparability, all methods were used on one and the same instrument, which did not undergo any changes over the entire course of this study.

4.2 Experimental section

Reagents. Measured analytes originated from a calibration standard (502.2 Cal2000 Mega Mix, 2 g L^{-1}) purchased from Restek GmbH (Bad Homburg, Germany). Standards, dilution steps and samples were prepared in Analytical grade methanol (KMF Laborchemie, Lohmar, Germany) or lab water from a PURELAB Ultra Analytic water purification system (ELGA LabWater, Celle, Germany).

Hamilton syringes (Hamilton, Bonaduz, Switzerland) were used for transferring standards, Blaubrand® bulb pipettes (Brand, Wertheim, Germany) were used for transferring water and 20-mL headspace vials with screw caps obtained from BGB Analytik AG (Boeckten, Switzerland) were used for sample storage.

Standard and sample preparation. From the obtained calibration mix, a methanolic standard solution with a concentration of 1 mg L^{-1} was prepared and stored in a vial without headspace in the refrigerator at 4°C. For minimizing the amount of methanol in the final samples, dilution steps were prepared in lab water, resulting in a final, maximal methanol concentration of 1 mg L^{-1}.

Samples were directly prepared in 20-mL screw cap headspace vials with silicone/PTFE septa (BGB Analytik, Boeckten, Switzerland) by adding the required volumes of the standard dilutions to 10 mL of lab water, resulting in approx. 10 mL headspace per sample vial.

Agitation of the samples, which were processed by the PAL RTC autosampler (see next section) was carried out using self-constructed stir bars, which were prepared from 1.5 x 10 mm magnetic iron cylinder bolts according to DIN EN ISO 2338 enclosed in fused silica. These were used in conjunction with an also self-constructed agitation station, which was based on an IKA-Mag RCT basic (IKA-Werke GmbH & CO KG, Staufen, Germany).

The samples processed by the Shimadzu HS-20 headspace-sampler, did not require stir bars since this instrument performs agitation by a carousel, in which the vials are rapidly moved in a circular manner. Details concerning the sample processing by the two autosamplers which were used in this study can be found in the supporting information part of this chapter (SI).

Extraction, Desorption and Cleanup Parameters. In order to achieve proper comparability of the measurements in this study, all samples were subjected to similar pre-conditioning and extraction parameters. Prior to analysis, all sample vials were stored in their respective trays at room temperature (23°C). For the PAL-based extraction methods, these vials were transferred to the agitation station, where the samples were stirred at 500 rpm and heated to 60°C for an initial equilibration time of 10 min.

In case of the static enrichment techniques, SPME fibers or PAL SPME Arrows were preconditioned in the needle heater station at 200°C under a stream of nitrogen 5.0 (Air Liquide, Oberhausen, Germany) simultaneous to the first 5 min of sample equilibration time. Further details such as the used fiber types and sorption phase materials can be found in the SI.

After preconditioning, the sample vials septa were pierced by the respective PAL tool, in order for the static sampling or enrichment processes to occur. Sample extraction in case of SPME and PAL SPME Arrow was carried out for 20 min. Thereby, all syringe temperatures were constantly kept at 60°C and ITEX sorption phases were kept at 40°C.

Thermal desorption of SPME fibers and SPME Arrows was carried out in the GC injector port at 250°C. ITEX sorption phases were heated to 300°C during desorption.

As sorption phase materials, Tenax TA and Tenax GR were used. Further ITEX parameters were as follows: Extraction gas volume: 1 mL, extraction gas flow (aspiration speed): 50 µL s^{-1}, pullup delay: 1.2 sec, number of extraction strokes: 60, desorption flow: 25 µL s^{-1}, desorption gas: 500 µL helium 5.0 aspired from the injector prior to heating the extraction phase to the desorption temperature.

Subsequent to desorption, SPME fibers and PAL SPME Arrows were again conditioned at the needle heater station for another 10 min at 200°C in order to avoid carryover.

Syringes and ITEX-phases were flushed by a stream of nitrogen 5.0 at approx. 20 kPa for the same period of time. The cleanup temperature of ITEX phases was thereby set to 300°C, while all syringes were kept at 60°C.

In case of the samples which were processed by the Shimadzu HS-20 headspace sampler, the method parameters were as follows: Oven (agitation carousel) temperature: 60°C, shaking level: 3 (of 5), equilibration time: 10 min, sample line and transfer line temperature: 200°C, pressurizing gas pressure: 60 kPa, pressurizing time: 2 min, pressure equilibration time and load equilibration time: 0.1 min, loading time: 0.5 min.

In case of using the trap functionality, the additional parameters were the standby temperature of the trap (25°C), its temperature during the enrichment of analytes (5°C) and the subsequent desorption (280°C), as well as the dry purge step, which was set to 1 min at 5°C with a nitrogen 5.0 flow of 50 kPa.

The multi-injection count was set to 5 of 10, which is a recommended setting for combining maximal sensitivity and repeatability according to the manufacturer. In each case, measurements were concluded by a needle purge time of 10 min.

GC/MS Instruments and parameters. All analyses were performed using a Shimadzu GCMS-QP2010 Ultra (Shimadzu Deutschland GmbH, Duisburg, Germany). Desorption of analytes was performed using a split/splitless injector, which was set to a temperature of 250°C and equipped with one of the following liners:

Either a Restek split/splitless liner with the following dimensions: 3.5 mm x 5.0 mm x 95 mm (BGB Analytik, Boeckten, Switzerland), or, in case of SPME and PAL SPME Arrow measurements, a 1.8 mm x 5.0 mm x 95 mm Restek Sky Arrow SPME Liner (also BGB Analytik).

Analyte separation was accomplished on a Restek Rtx®-VMS column (Restek GmbH, Bad Homburg, Germany), with an inner diameter of 0.25 mm, a length of 60 m and a film thickness of 1.4 µm. The range of retention times was between 5.9 min and 22.9 min for 1,1-dichloroethene and 1,2,3-trichlorobenzene (see Table 14), respectively.

The chosen chromatographic conditions enabled sufficient separation of the selected target compounds with an exemplary chromatogram being shown in Figure 37 of the SI.

Target compounds were an exemplary selection of 41 representative volatile organic carbon (VOC) analytes which were found suitable for this comparison study in preliminary measurements.

For thermal desorption and pre-column focusing of analytes from fibers in case of the static enrichment techniques, the injector was programmed to a flow of 1.5 mL min^{-1} Helium 5.0 (Air Liquide, Oberhausen, Germany) with a splitless time of 2 minutes at the beginning of the analysis sequence and a subsequent split ratio of 10:1. The starting temperature of the column oven was set to 35°C for 1.5 min, followed by a first rapid heating of 50°C min^{-1} up to 60°C, another ramp of 5°C min^{-1} to 190°C and a concluding hold time of 7 min.

The mass spectrometer (MS) was set to single ion monitoring (SIM) mode. Transfer line and ion source were both set to 250°C. The tuning was regularly carried out with FC43 (perfluorotributylamine, CAS 311-89-7) (BGB Analytik, Boeckten, Switzerland). Ionization of analytes was performed via electron impact ionization (acceleration voltage = 70 eV, emission current = 150 µa). For each compound, characteristic fragments were monitored. Isomeric substances were identified with the help of their specific retention times. Detailed SIM conditions are listed in Table 14. Analytes in identical segments were analyzed as part of the same SIM window due to similar retention times.

Table 14: GC/MS detection parameters for the investigated constituents of the Restek VOC Mega Mix for the comparison of several headspace techniques

Compound	Retention time	Segment	Quantifier ion (m/z)	Qualifier ions (m/z)
1,1-Dichloroethene	5.9	1	61	96,63
Dichloromethane	6.6	1	49	84,86
1,2-Dichloroethene (E)	6.8	1	61	96,63
1,1-Dichloroethane	7.5	1	63	65,83
1,2-Dichloroethene	8.2	2	61	96,63
2,2-Dichloropropane	8.5	2	41	77,79
Trichloromethane	8.5	2	83	85,49
Tetrachloromethane	8.8	2	117	119,121

1,1,1-Trichloroethane	8.8	2	97	61,99
1,1-Dichloropropene	8.9	2	75	77,110
Benzene	9.4	3	78	77,52
1,2-Dichloroethane	9.6	3	62	64,49
Trichloroethylene	10.2	3	95	130,132
Dibromomethane	10.9	3	93	95,174
1,3-Dichloropropene (E)	11.0	3	63	62,41
1,3-Dichloropropene (Z)	12.1	3	75	77,49
Toluene	12.6	3	91	92,65
Tetrachloroethylene	13.4	4	129	131,75
1,1,2-Trichloroethane	13.7	4	83	97,61
1,3-Dichloropropane	14.1	4	129	127,48
Ethylbenzene	14.3	4	41	76,78
1,1,1,2-Tetrachloroethane	14.7	4	107	109,81
m-Xylene	15.8	5	91	77,112
o-Xylene	15.9	6	131	133,117
Styrene	16.1	6	91	106,105
Isopropylbenzene	17.1	6	91	106,105
n-Propylbenzene	17.3	6	104	78,103
4-Chlorotoluene	17.9	6	105	120,79
1,2,3-Trimethylbenzene	19.0	7	91	77,120
2-Chlorotoluene	19.1	7	83	85,61
sec-Butylbenzene	19.4	7	91	126,89
1,2,3-Trimethylbenzene	19.5	7	105	120,77
p-Isopropyltoluene	19.8	7	91	126,65
tert-Butylbenzene	20.3	8	119	91,41
1,3-Dichlorobenzene	20.5	8	105	120,77
1,4-Dichlorobenzene	20.8	8	105	134,91
n-Butylbenzene	21.2	8	119	91,134
1,2-Dichlorobenzene	21.5	8	146	111,148
1,2,4-Trichlorobenzene	21.7	8	180	182,74
Naphthalene	22.4	9	128	102,51
1,2,3-Trichlorobenzene	22.9	9	180	182,74

4.3 Results and discussion

Estimation of extraction efficiency. Effectiveness of the specific techniques was estimated from the amount of analyte that is transferred to the GC injector under the assumption of full equilibration during extraction.

Table 15 displays the calculated amounts of exemplary analytes that are introduced into the GC for all methods. The resulting values for the ITEX technique were tentatively calculated assuming full equilibration which will hardly be reached practically due to losses of analytes, which are remobilized from the sorption phase and flushed back into the sample vial during dispensing. More details concerning the calculation of the presented values can be found in the SI.

Table 15: Exemplary transferred amounts of analytes (in pg) calculated for each sample preparation technique for an initial analyte concentration of 10 ng L^{-1} in the liquid sample (100 pg of each analyte per vial) according to the Abraham model[79,80,56]

Compound name	Method name (sorption phase material if applicable) and extracted amounts (pg)					
	Syringe	HS-20 Loop	HS-20 Trap (Tenax TA)	SPME (0.6 µL PDMS)	PAL SPME Arrow (10.2 µL PDMS)	ITEX DHS (160µL Tenax TA)
1,1-Dichloroethene	6.9	6.8	100	95.0	100	100
Methylene Chloride	2.5	2.4	88.3	69.3	100	100
1,2-Dichloroethene (E)	5.4	5.2	99.9	60.1	100	100
1,1-Dichloroethane	4.1	4.0	98.5	94.9	100	100
1,2-Dichloroethene	3.2	3.1	94.7	48.7	99.0	100
2,2-Dichloropropane	2.7	2.7	91.1	92.7	100	100
Chloroform	3.5	3.4	96.5	90.3	100	100
Carbon Tetrachloride	7.0	6.8	100	3.0	34.4	90.2
1,1,1-Trichloroethane	6.1	5.9	100	19.0	80.8	99.8
Benzene	4.1	4.0	98.5	39.3	97.7	100
1,2-Dichloroethane	1.4	1.4	67.3	42.0	98.1	99.9
Trichloroethylene	5.5	5.3	99.9	28.0	87.9	100
Dibromomethane	0.7	0.7	42.2	28.7	95.6	98.7

Toluene	4.4	4.3	99.1	18.5	91.6	96.8
Tetrachloroethylene	6.6	6.4	100	12.9	87.1	94.5
1,1,2-Trichloroethane	1.3	1.2	62.0	14.3	88.5	95.2
1,3-Dichloropropane	1.0	1.0	54.5	23.4	93.9	97.9
Ethylbenzene	5.4	5.2	99.9	8.9	62.8	97.6
1,1,1,2-Tetrachloroethane	0.4	0.4	23.1	8.6	80.5	90.8
m-Xylene	4.5	4.4	99.3	7.7	78.5	90.5
o-Xylene	3.8	3.7	97.6	28.3	88.0	100
Styrene	3.4	3.3	95.7	7.2	77.3	89.0
n-Propylbenzene	5.1	4.9	99.8	3.1	35.5	90.7
4-Chlorotoluene	2.6	2.6	89.9	3.8	62.7	79.5
1,2,3-Trimethylbenzene	4.4	4.3	99.1	1.9	45.4	65.4
sec-Butylbenzene	4.1	4.0	98.6	2.3	49.7	69.2
1,2,3-Trimethylbenzene	4.1	4.0	98.7	3.0	57.1	75.3
p-Isopropyltoluene	4.1	4.0	98.6	1.9	45.3	65.3
1,3-Dichlorobenzene	1.9	1.9	79.2	10.7	67.7	98.3
1,4-Dichlorobenzene	2.0	1.9	79.8	2.4	51.4	70.6
n-Butylbenzene	5.1	4.9	99.7	1.5	38.7	58.7
1,2-Dichlorobenzene	3.0	2.9	92.9	4.3	43.3	93.4
1,2,4-Trichlorobenzene	2.1	2.0	81.6	2.1	26.4	85.9
Naphthalene	0.9	0.8	47.1	0.6	19.4	35.0

In order to validate the calculated values with measured results, the extraction yields presented in Figure 36 were determined exemplarily according to the depletion method of Zimmermann et al.[58] using samples with an initial concentration of 1 µg L^{-1}. Detailed results and further explanations for these measurements can be found in the SI. The means of the calculated extraction efficiencies from Table 15 are indicated as well for straightforward comparison of the results.

Note that the depletion method for extraction yield determination cannot be applied to techniques that discard large portions of the sample headspace such as the HS-20 Loop. In this system, static sampling is conducted by venting the pressurized headspace through the loop and ultimately into the vent.

Therefore, a larger portion of the analytes is abstracted from the sample vial than is actually transferred into the injector. Since the depletion method calculation is based on declining results with each consecutive analysis of the same sample, a closed system in terms of the sample vessel is an indispensable prerequisite.

The headspace gas which is abstracted by the syringe from the pressurized sample vial equilibrates its pressure with the ambient lab air as soon as the syringe needle is removed from the vial, resulting in a loss of headspace gas. Ultimately a smaller amount of headspace gas is transferred to the injector than the one which was abstracted from the sample vial. This may explain why the static syringe is the only technique whose measured extraction yields are actually larger than the previously calculated ones.

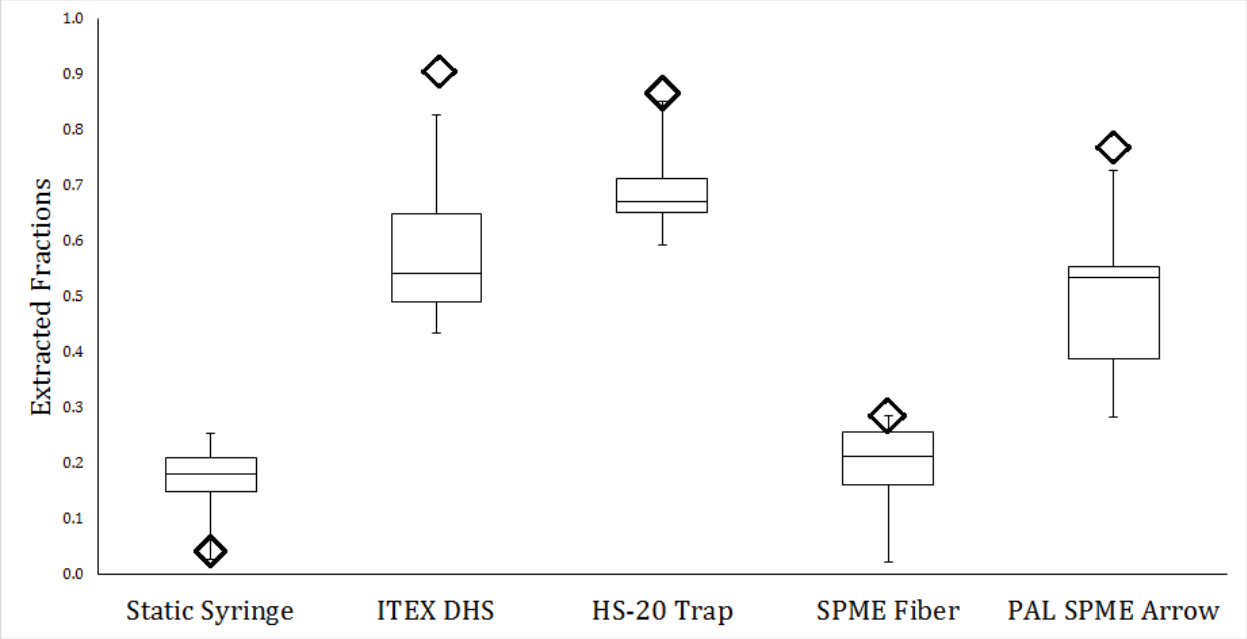

Figure 36: Boxplots of mean extraction yields[58] for exemplary compounds specified in the SI, determined for an initial analyte concentration of 1 µg L^{-1}. Whiskers represent total minima and maxima of the data which are shown in detail in the SI. Diamonds indicate calculated, mean extracted fractions based on the values displayed in Table 15.

Calculated and measured results correlate fairly well, with a positive bias for the calculated results except for the syringe technique as already discussed. In case of the enrichment techniques, this bias may have been caused by an incomplete equilibration of analyte concentrations between sample solution, headspace gas and the sorption phase materials. As discussed before, the ITEX enrichment process hardly ever reaches a full equilibrium, since the dispensing steps always remobilize analytes from the sorption phase and transfer them back into the sample vial. In addition the volume of gas that is cycled through the extraction phase in case of this technique is limited to 1.3 mL and typically set to 1 mL.

Even an exhaustive depletion of this volume would therefore only result in a depletion of one tenth of the overall sample headspace (10 mL in this work). The next extraction stroke would then aspirate diluted headspace gas with only 90% of the equilibrated analyte headspace concentration. This continuous dilution will proceed until the transfer of analytes from the sample solution to the headspace outweighs it. The effect of this dilution is therefore inversely proportional to analyte volatility. These two aspects may explain why the ITEX technique displays the largest discrepancy between calculated and measured extraction yields: It would need very long extraction times, i.e., number of extraction strokes, in order to reach a fully equilibrated three-phase system.

Generally speaking, complete equilibration - also for SPME and PAL SPME Arrow - requires an infinite amount of time to establish and is therefore never fully obtained. Nevertheless, longer extraction times may have decreased the discrepancy between calculated and measured extraction yields. The displayed results can also be taken as an indicator that the enlarged sorption phases of PAL SPME Arrow would have benefitted from a prolonged extraction time to a larger extent than the classical SPME fibers. For the trap technique, the obtained results may indicate breakthrough of analytes through the sorption phase, which are - in contrast to ITEX - irreversibly lost.

The plausibility of the obtained extraction yields can be confirmed by comparison to literature. For the classical SPME fiber, e.g., Zimmermann et al. found extraction yields in the range of 3.2 to 27.8%[58]. While these values were generated via immersive extraction for different analytes (pesticides), the general order of magnitude of the results remains comparable.

Lamani et al. reported extraction yields for iodobenzenes between 66 and 84% using headspace SPME [81]. These results may indicate that adsorption to DVB in the PDMS/DVB coating used as sorption phase in this study, contributes considerably to the overall extent of sorption.

Comparable ratios were for example found for the solid-phase dynamic extraction technique (SPDE), which is superior to SPME in its sorption phase volumes (approx. 4.5 µL of phase volume) with a mode of action similar to ITEX[82]. The same holds true for PAL SPME Arrow for which we have determined extraction yields in the range of 17.5 to 65.7% for PAHs extracted via immersion from aqueous samples[7]. While the range of these results is larger than of those presented in this paper, the overall magnitude of the results also fits in this case.

For the ITEX technique, literature contains slightly smaller extraction yields. Laaks et al. found a range of 7 to 55%[8] for this technique. The smaller yields were however found for less volatile compounds such as 2-methylisoborneol or geosmin. The results generated for the trap technique are in good agreement with literature data such as those found by Schulz et al., who reported 35 to 55 times higher extraction yields for the trap technique compared to static headspace sampling[77].

Method validation. Major method validation data are discussed in the following. Further details such as results for alternative sorption phase materials can be found in the electronic supplementary material (SI).

Table 16 shows measurement repeatability in terms of the relative standard deviations (RSDs) for the exemplary compounds. Values for enrichment techniques are given at a concentration of 20 ng L^{-1} and values for static sampling methods are given at a concentration of 200 ng L^{-1}.

Table 16: RSDs for individual headspace technique, determined from five replicate measurements. RSDs for the loop and syringe method were determined at 200 ng L^{-1} while all other RSDs where determined at 20 ng L^{-1}. Slashes indicate insufficient certainty of the corresponding peak identification for specific compounds and techniques.

Compound-name	Loop RSD (%)	Syringe RSD (%)	SPME RSD (%)	Arrow RSD (%)	Trap RSD (%)	ITEX RSD (%)
1,1-Dichloroethene	12.9	25.5	11.9	/	1.7	1.8
Methylene Chloride	10.5	4.3	2.1	3.6	1.4	2.6
1,2-Dichloroethene (E)	9.3	8.6	/	/	0.7	6.4
1,1-Dichloroethane	8.4	9.1	/	/	/	3.6
1,2-Dichloroethene	9.1	5.4	/	4.6	0.5	2.8
2,2-Dichloropropane	20.2	7.8	/	/	/	5.6
Chloroform	8.8	6.9	15.2	/	4.5	3.1
Carbon Tetrachloride	9.1	10.0	12.8	0.7	0.8	4.3
1,1,1-Trichloroethane	8.4	8.6	13.1	/	9.8	6.2
1,1-Dichloropropene	9.2	7.3	/	/	3.0	8.6
Benzene	8.7	5.2	/	8.0	2.2	2.4
1,2-Dichloroethane	8.0	5.1	2.2	/	1.6	2.5
Trichloroethylene	8.6	6.3	8.9	1.1	9.7	5.8
Dibromomethane	5.2	9.4	/	1.3	/	5.4
1,3-Dichloropropane (E)	5.5	7.1	/	/	7.3	4.5
1,3-Dichloropropene (Z)	9.4	29.1	/	/	/	5.8
Toluene	8.2	5.4	18.1	3.4	0.5	2.9
Tetrachloroethylene	7.9	8.6	4.4	2.4	2.0	7.1
1,1,2-Trichloroethane	6.1	6.5	14.1	/	/	6.0
Propane, 1,3-dichloro-	9.2	7.6	7.0	2.4	/	7.0
Ethylbenzene	7.9	17.9	26.8	7.7	2.2	5.4
1,1,1,2-Tetrachloroethane	9.8	6.2	7.7	5.4	3.6	7.0
m-Xylene	8.7	6.3	4.0	0.9	0.9	3.6
o-Xylene	8.1	6.9	2.5	7.5	1.7	4.6
Styrene	8.1	7.4	8.8	4.8	2.8	2.1
Isopropylbenzene	7.5	6.7	2.5	3.5	2.1	3.4
n-Propylbenzene	7.9	7.9	2.4	3.0	1.4	2.9
4-Chlorotoluene	8.9	7.3	1.8	2.2	1.2	6.9
1,2,3-Trimethylbenzene	8.2	7.7	1.8	8.8	1.7	4.0
2-Chlorotoluene	9.4	5.5	1.6	10.0	1.3	1.8
sec-Butylbenzene	7.3	8.3	/	7.2	1.3	3.8

1,2,3-Trimethylbenzene	8.2	5.5	/	4.8	1.4	3.1
p-Isopropyltoluene	7.1	9.6	3.4	7.2	2.3	2.7
tert-Butylbenzene	7.4	8.6	1.3	4.8	1.3	3.9
1,3-Dichlorobenzene	9.3	11.0	1.3	5.3	1.2	0.5
1,4-Dichlorobenzene	9.2	4.7	0.3	3.7	1.8	4.7
n-Butylbenzene	9.0	4.3	2.2	4.9	1.5	4.5
1,2-Dichlorobenzene	8.9	7.9	0.2	4.8	3.0	2.4
1,2,4-Trichlorobenzene	11.0	5.7	0.3	4.7	4.5	2.4
Naphthalene	6.6	4.4	10.4	3.7	/	3.2
1,2,3-Trichlorobenzene	8.5	3.8	3.1	/	/	2.5
Mean over all analytes	**8.8**	**8.2**	**14.8**	**4.6**	**2.5**	**4.1**

Obtained RSDs were suitable and mostly homogeneous for each technique with the classical SPME fiber showing the largest deviations, which are also slightly higher than in some previous studies where typical ranges between 3 and 7% have been reported [11,76]. Similar ranges were also presented for headspace-SPME in a collection of applications[63].

The method detection limits (MDLs) presented in Table 17 were determined at different concentrations depending on the mode of action of the individual method and the S/N≈3 criterion: The MDLs of enrichment techniques were determined at a concentration of 20 ng L^{-1} and those of the static sampling based methods at 200 ng L^{-1}.

Calculation was thereby similar to the EPA method [83], with the difference that the measurements were repeated only five times. This method of MDL determination is very reliant on the measurement repeatability, which is therefore more important than the instrumental sensitivity in terms of signal to noise ratios at certain concentration levels.

Table 17: Method detection limits for individual headspace techniques, determined from five replicate measurements. MDLs for the loop and syringe method were determined at 200 ng L^{-1} while all other MDLs where determined at 20 ng L^{-1}. Slashes indicate insufficient certainty of the corresponding peak identification for specific compounds and techniques.

Compound-name	Loop MDL (ng L^{-1})	Syringe MDL (ng L^{-1})	SPME MDL (ng L^{-1})	Arrow MDL (ng L^{-1})	Trap MDL (ng L^{-1})	ITEX MDL (ng L^{-1})
1,1-Dichloroethene	67.5	143	52	/	1.8	1.6
Methylene Chloride	235	29.5	4.1	1.1	6.5	2.9
1,2-Dichloroethene (E)	53.5	48.4	/	/	0.7	7.0
1,1-Dichloroethane	45.6	168	/	/	/	3.7
1,2-Dichloroethene	50.3	41.0	/	3.8	1.7	6.4
2,2-Dichloropropane	200	67.9	/	/	/	4.0
Chloroform	47.2	36.5	13.9	/	6.9	5.6
Carbon Tetrachloride	48.6	59.2	19	2.0	0.9	7.9
1,1,1-Trichloroethane	44.9	53.9	21.1	/	9.6	9.1
1,1-Dichloropropene	47.3	47.7	/	/	3.3	2.8
Benzene	47.9	65.7	/	2.3	2.6	8.6
1,2-Dichloroethane	43.7	24.1	14.6	/	1.8	7.0
Trichloroethylene	45.5	39.7	23.2	3.2	9.5	5.1
Dibromomethane	23.0	37.7	/	0.9	/	8.1
1,3-Dichloropropane (E)	25.3	35.8	/	/	7.4	4.0
1,3-Dichloropropene (Z)	57.7	/	/	/	/	7.4
Toluene	45.3	39.0	30.9	4.1	0.6	6.6
Tetrachloroethylene	42.7	48.9	13.2	1.9	2.3	8.2
1,1,2-Trichloroethane	29.1	31.7	13.0	/	/	4.9
Propane, 1,3-dichloro-	41.4	30.8	33.6	4.8	/	8.6
Ethylbenzene	43.4	122	79.6	3.4	2.6	4.2
1,1,1,2-Tetrachloroethane	44.5	52.5	3.4	3.1	3.4	5.2
m-Xylene	48.7	32.1	20.4	0.7	1.2	2.4
o-Xylene	44.8	59.9	13.8	3.3	2.2	4.0
Styrene	45.1	63.9	10.0	4.8	3.6	3.6
Isopropylbenzene	40.0	54.1	3.2	4.1	2.5	6.9
n-Propylbenzene	43.6	68.8	3.1	4.0	1.8	4.1

4-Chlorotoluene	48.6	63.4	2.1	3.4	1.3	4.1
1,2,3-Trimethylbenzene	45.4	31.0	2.1	3.0	2.1	3.0
2-Chlorotoluene	55.0	42.1	15.0	3.5	1.4	3.0
sec-Butylbenzene	38.3	75.2	/	3.3	1.6	3.8
1,2,3-Trimethylbenzene	47.2	42.2	/	4.3	1.6	0.9
p-Isopropyltoluene	37.2	86.7	4.4	4.1	2.7	4.2
tert-Butylbenzene	40.8	73.2	1.7	2.9	1.6	4.3
1,3-Dichlorobenzene	51.4	99.7	1.5	2.3	1.4	2.2
1,4-Dichlorobenzene	51.1	27.2	3.1	2.1	2.1	2.0
n-Butylbenzene	52.2	25.5	2.76	3.2	1.7	3.4
1,2-Dichlorobenzene	45.4	43.7	7.9	2.0	3.1	2.2
1,2,4-Trichlorobenzene	60.6	26.9	0.5	4.0	4.9	1.8
Naphthalene	35.0	26.1	11.0	3.9	/	5.9
1,2,3-Trichlorobenzene	45.0	25.0	4.0	/	/	2.3

Detection limits are approx. one order of magnitude better for the enrichment techniques. This general trend is contrasting to some literature data where mostly similar detection limits for the enrichment technique SPDE and the syringe headspace technique were found [82].

However, other studies found even larger differences between static sampling and enrichment techniques. For the analysis of BTEX from aqueous samples headspace-SPME and the syringe technique were compared resulting in improved detection limits by a factor of up to 250[11]. For volatile flavor compounds a ratio of roughly 100:1 between the detection limits achieved via SPME and static syringe headspace was reported[75].

The smaller factor of 10 between the MDLs of static and enrichment techniques reported in this work, however, seems plausible if one compares it with the calculated and determined extraction yields of the methods discussed above.

Calculated and measured results unveiled comparatively smaller extraction yields for the static enrichment techniques SPME and PAL SPME Arrow compared to the dynamic techniques.

These differences are also visible in the resulting MDLs and are more pronounced in case of the classical SPME fiber. The higher extraction yields of PAL SPME Arrow are, also apparent in the resulting lower MDLs for this technique compared with SPME.

Mean RSDs and MDLs obtained with the HS-20 in its trap mode and ITEX DHS are very similar. The obtained MDLs are in good agreement with data reported in literature for single methods, as is exemplarily shown for ethylbenzene in Table 18. Discrepancies to the MDLs in this study can be explained by the different methods for MDL determination.

Table 18: Comparison of the MDL values found during the comparison of several headspace techniques with literature data for ethylbenzene

Method	This study (MDL in ng L^{-1})	Literature data (MDL in ng L^{-1})	Literature method of MDL determination	Source
Syringe	122	330	*	[84]
SPME	80	5	German DIN[85]	[86]
ITEX	4.2	2	MDL [59]	[8]
Trap	2.6	5.6	Not stated	[87]

* LOD = 3 x S_{blank} /slope of the calibration graph, where S_{blank} is the standard deviation of ten blank values

The plausibility of the presented results can further be demonstrated by comparison with literature data obtained with related techniques. An example for this is the needle-trap, a method which is similar to the ITEX system[88]. Resulting detection limits for VOC analytes with this technique are in the range of 60 to 10 ng L^{-1}[89] and therefore higher than those achieved via ITEX in this study. This is in agreement with the smaller sorption phase volumes of up to 4 µL for this technique[2].

As another alternative technique for headspace analysis, Purge & Trap is based on actively purging volatile analytes out of an aqueous sample by a constant stream of inert gas that is directed through the liquid sample.

The transition of analytes from the liquid into the gaseous phase is therefore facilitated and most instruments permit the use of sample volumes that are larger than in case of the standard 20-mL headspace vials used in this study. Resulting detection limits e.g. in case of BTEX are in the range of 22 to 2 ng L^{-1}[84] and therefore comparable to the results that could be achieved with the ITEX system in this study. Thus, even when very low MDLs are required, the dedicated purge & trap systems rarely offer clear advantages, corroborating previous results[8].

In order to enable a broader evaluation of the presented results in comparison with literature data, Table 19 summarizes MDLs reported in previous studies using mostly a single extraction technique.

For comparing these values it should be taken into account that determination methods for the detection limits often deviate among studies. Finally, some of the studies shown here for comparison involved different matrices such as urine or were carried out using different detection options such as electron capture detection (ECD)[90]. For these reasons, the presented values should not be used for comparing absolute results but rather general trends and relative differences between the methods, which generally correlate well with the data presented in this work.

Table 19: Comparison of detection limit ranges shown in this work and in various literature sources. Sample matrices and MDL determination methods are indicated as well (n.s. = not specified), a) = MDL according to EPA[59], b) = baseline standard deviation x 3, c) = according to German standard procedure (DIN 32645)[85], d) = according to IUPAC, e) = extrapolated from standard curve

Method name	This work	Sources with reported detection limits in ng L^{-1}										
		[91]	[63]	[11]	[13]	[8]	[90]	[92]	[77]	[87]	[65]	[93]
Syringe	25-143	/	/	1-2E+3	/	/	25-53	/	66-570E+3	/	/	/
Loop	25-168	/	/	/	/	/	/	/	/	/	8-20E+3	5-20E+3
SPME PAL	0.5-79.6	5-50	2-550	80-600	/	/	/	8-12	/	/	2-26E+3	/
SPME Arrow	0.7-4.9	/	/	/	/	/	/	/	/	/	/	/
Trap	0.6-9.6	/	/	/	/	1-10	/	/	7-149E+3	0.5-91	/	/
ITEX	0.9-9.1	/	/	/	28-799	1-70	/	/	/	/	/	/
Sample matrix	Lab water	Spirit	Waste-water	Water	Lab water	Lab water	Urine	Meconium	Spirit	Water	Beer	Wet Rice
MDL method	a)	b)	n.s.	b)	a)	a)	n.s.	n.s.	c)	d)	e)	b)

4.4 Conclusions

Considering the cost, footprint, complexity and susceptibility to contamination of dedicated trap sampling or purge & trap instruments, microextraction techniques such as the PAL SPME Arrow or ITEX DHS may be a more efficient choice for many analytical applications. While purge & trap systems aim for an exhaustive extraction of analytes and potentially exhibit a mean extraction yield > 90%[15,9], this would correspond to an improvement at the most of a factor two compared to, e.g., ITEX DHS.

While the RSD values of all compared techniques were sufficiently good for all analytes, especially the determined MDL values allow for an effective assessment of the possible operating ranges of the discussed method classes in terms of target compound concentrations.

The syringe and loop methods as the static sampling types produce adequate results down to approx. 100 ng L^{-1}. They are therefore sufficient for many routine headspace analysis methods such as the U.S. Pharmacopeial Convention (USP) method 467 for the analysis of residual solvent amounts in pharmaceuticals, which requires detection in the mg L^{-1} range[94].

Enrichment methods achieve detection limits in the low ng L^{-1} range. The differences between static and dynamic enrichment methods are mostly insignificant in terms of MDL and RSD values.

Similarly, the different sorption characteristics of varied phase materials in case of PAL SPME Arrow caused only minor variations of results (see SI for detailed data).

The influences of method selection and setup decisions on the final outcome of a headspace analysis can therefore be summarized as follows: Method class (mode of action) > Method characteristics (dynamic/static) > Sorption phase material selection (for enrichment techniques). This ranking, however, depends on the properties of the target compounds and may change when analyzing analytes with different properties such as high polarity or the capability of specific molecular interactions.

4.5 Supporting information

Vial volume calculation. The precise amount of headspace per vial was determined with a Sartorius AC210S precision balance (Sartorius AG, Göttingen, Germany) and 25 sample vials that were measured in an empty and completely water-filled state at 25°C. The mean value of the vial volumes was determined to be 20.395 (± 0.118) mL at these conditions.

Under practical measuring conditions however, water samples were heated to 60°C, slightly expanding the liquid volume. This in total results in 10.141 (± 0.059) mL of water being present in the vials alongside 10.254 (± 0.059) mL of headspace.

Sample processing. Samples were processed by two different autosamplers. The first one was a PAL RTC (CTC Analytics AG, Zwingen, Switzerland), which was equipped with automatically changeable tools for the following techniques: A syringe headspace tool for static headspace analysis, an ITEX DHS tool for dynamic extraction and a SPME as well as a PAL SPME Arrow tool as static enrichment alternatives. The syringe headspace tool was outfitted with a standard 2.5 mL gastight, HD-type Hamilton syringe (Hamilton, Bonaduz, Switzerland). The ITEX DHS tool was equipped with a Tenax TA sorption phase, which was obtained from BGB Analytik.

Concerning the static enrichment techniques, A 100 µm PDMS fiber supplied by CTC Analytics and the new PAL SPME Arrow was used in a 1.5 mm wide variant, with a cylindrical sorption phase possessing a thickness of 250 µm, a length of 20 mm, a phase volume of approx. 15 µL and a surface area of approx. 85 mm^2. For successful implementation of the Arrow's larger diameter in contrast to traditional SPME, the openings of the GC injector and the fiber conditioning station were widened to 1.8 mm.

The second autosampler used in this work is a Shimadzu HS-20 automated headspace-sampler, which has two different variants and corresponding modes of function. In its basic variant ("Loop"), it is solely capable of static headspace analysis via a loop-based system. The so called "Trap" variant, which was also used in this study, is in addition also capable of enriching analytes on a polymeric sorption phase. It does so in intervals, which are interrupted by short periods of time during which the sample vial is re-pressurized and the sample headspace re-equilibrated.

Independent of whether the system is set to its loop or trap functionality, abstraction of the headspace is always carried out when the sample vial is transported up and out of the agitation carousel by a pushing rod. This presses the vial against a downward-facing syringe that pierces through its septum, connecting the sample headspace to the tempered Sulfinert®-treated stainless steel capillaries and valves of the instrument. Afterwards, the pressure inside the vial is equilibrated to a constant value for a defined amount of time, prior to venting the headspace either through the loop, or the trap. The latter consists of a 1/8 inch steel capillary filled with Tenax TA (changeable if desired), which can be cooled down to -20°C for an improved enrichment of extremely volatile analytes. Subsequently, the trap may be subjected to a dry purge step, using a stream of nitrogen in order to remove residual amounts of water prior to GC/MS analysis. It is then heated to the desired desorption temperature and analytes are purged from the trap into the GC injector, with reciprocal flow direction of the preceding enrichment step for improved peak shapes.

Calculation of transferred amounts. The first step in calculating the effectively transferred amounts of exemplary analytes by each technique is to determine their amounts in the equilibrated sample headspace, which is the starting point of all headspace analyses. For this, first the air-water partitioning constant Kaw is required. Partitioning between water and air is strongly temperature-dependent, which can be taken into account by a simplified and generalized Van't Hoff equation (1)[52]:

$$logK_{aw} = A - \frac{B}{T} \qquad (1)$$

Thereby, *A* and *B* are curve fitting parameters which were obtained from literature[52] while *T* is the temperature (333.15 K). The obtained K_{aw} value can then be used with equation (2)[4] in order to determine the analyte concentration that is present in the sample headspace under equilibrium conditions (c_{hs}):

$$c_{hs} = \frac{c_{s0}}{\left(\frac{1}{ß} + \frac{1}{K_{aw}}\right)} \qquad (2)$$

Where c_{s0} is the initial analyte concentration in the sample solution and ß is the phase ratio of the sample solution to the sample headspace (V_s/V_{hs}).

The density of water is decreased at elevated temperatures, thus its' volume at 60°C was calculated as 10.14 mL. Determined individual K_{aw} and c_{hs} values are listed in Table 20. Note that the values for sec-butylbenzene and n-butylbenzene were calculated for the structurally similar compounds cumene and propylbenzene respectively, due to lack of descriptor data (curve fitting parameters A and B for equation (1)).

Since data for 1,2,3-trichlorobenzene were not available either, 1,2,4-trichlorobenzene remained as sole representative of the trichlorobenzenes in these preliminary calculations.

Table 20: Individual K_{aw} and c_{hs} values for the exemplary compounds used for the comparison of several headspace techniques, calculated for a c_{s0} of 10 ng L^{-1} using given curve fitting parameters A and B according to Staudinger et al.[52]

Compound	A	B	K_{aw} (60°C)	c_{hs} (ng L^{-1})
1,1-Dichloroethene	5.397	1586	4.33	8.31
Methylene Chloride	4.561	1644	0.42	3.00
1,2-Dichloroethene (E)	5.247	1669	1.73	6.45

1,1-Dichloroethane	4.461	1498	0.92	4.86
1,2-Dichloroethene	4.464	1559	0.61	3.82
2,2-Dichloropropane	4.878	1730	0.48	3.29
Chloroform	5.343	1830	0.71	4.19
Carbon Tetrachloride	5.736	1689	4.64	8.42
1,1,1-Trichloroethane	5.163	1588	2.49	7.28
Benzene	5.053	1693	0.94	4.90
1,2-Dichloroethane	4.434	1705	0.21	1.72
Trichloroethylene	5.874	1871	1.81	6.56
Dibromomethane	3.661	1556	0.10	0.89
Toluene	5.271	1745	1.08	5.27
Tetrachloroethylene	6.394	1955	3.36	7.87
1,1,2-Trichloroethane	5.219	1989	0.18	1.51
1,3-Dichloropropane	3.888	1577	0.14	1.25
Ethylbenzene	6.541	2100	1.73	6.45
1,1,1,2-Tetrachloroethane	2.429	1255	0.05	0.44
m-Xylene	5.204	1713	1.15	5.44
o-Xylene	5.064	1719	0.80	4.51
Styrene	5.628	1935	0.66	4.02
n-Propylbenzene	4.587	1471	1.48	6.08
4-Chlorotoluene	3.89	1409	0.46	3.17
1,2,3-Trimethylbenzene	5.125	1697	1.07	5.26
sec-Butylbenzene	3.774	1265	0.95	4.93
1,2,3-Trimethylbenzene	4.329	1448	0.96	4.97
p-Isopropyltoluene	3.774	1265	0.95	4.93
1,3-Dichlorobenzene	2.436	986	0.30	2.32
1,4-Dichlorobenzene	2.649	1054	0.31	2.36
n-Butylbenzene	4.587	1471	1.48	6.08
1,2-Dichlorobenzene	7.045	2436	0.54	3.54
1,2,4-Trichlorobenzene	4.381	1622	0.33	2.47
Naphthalene	6.058	2332	0.11	1.03

In order to assess the efficacy of the individual headspace-sampling techniques, the amount of analytes that is transferred to the GC injector in each case is to be determined.

The most straightforward example in this context is the static syringe headspace technique, which transfers 1 mL of sample headspace containing the calculated analyte concentrations. During transfer, it however permits the headspace gas to equilibrate its pressure with the ambient lab air, which has to be taken into account during calculation of transferred analyte amounts.

During this equilibration, the headspace inside the syringe expands and partially leaves the syringe, resulting in a loss of analytes. Diffusive exchange is also possible, but probably negligible due to the short amount of time required for transferring the syringe from the sample vial to the injector (approx. 5 sec).

After equilibration at 60°C, the pressure inside the sample vials can be calculated as 121 kPa. From the ideal gas law a loss of analytes equaling approx. 16.5 % can then be estimated accordingly. The approximated, effectively transferred analyte amounts are given in the main paper. The same calculation can be done for the Shimadzu HS-20 in its' loop mode, which was set to an equilibration pressure of 161 kPa inside the sample vial. In order to fill the sample loop with a reproducible amount of analytes, the latter pressure is vented through this loop and a subsequent valve, regulating the remaining pressure inside the system in a controlled manner to 50 % of the relative original equilibration pressure - in this case 131 kPa.

A special case, however, is the trap mode of the Shimadzu HS-20. Since the sorption phase volume is comparatively large in this instrument, an exhaustive extraction of analytes from the internal gas flow inside the instrument can be assumed. The effectively retained analyte amounts are therefore directly correlated to the volume of headspace gas that passes through the trap. The corresponding values given in the main paper also take into account the depletion of analytes in the sample vial with each consecutive extraction sequence.

Calculation of effectively extracted amounts is not reliably possible for the ITEX technique, since the flow of headspace gas is not occurring in a single direction, but in alternating steps of aspiration and dispensing.

Furthermore, solute descriptors for Tenax TA were not available, so that solute descriptors according to the Abraham model[56] for the similar polymer PDMS were tentatively used in order to describe the ITEX DHS technique (see calculations for SPME and PAL SPME Arrow below).

While the aspiration through the sorbent bed leads to an enrichment of analytes, the subsequent dispensing step always includes a portion of analytes being released from the sorption phase and transferred back into the sample vial. This bidirectional extraction may require comparatively long timeframes depending on target compounds and desired exhaustiveness of the extraction[74] and complicates estimation of effectively retained analyte amounts per extraction cycle.

In case of the enrichment techniques, the extraction efficacy of distribution-based materials such as polydimethylsiloxane (PDMS) is predictable by their phase volumes and the individual analyte's tendency to transfer out of the sample matrix into the headspace and ultimately into this polymer. This tendency can be quantitatively described using solute descriptors according to the Abraham model[56] which can be found in Table 21. The corresponding distribution coefficients for the individual compounds were then calculated from equation (3):

$$logK_{fs} = 0.246 + 0.568 \times E - 1.305 \times S - 2.565 \times A - 3.928 \times B + 3.573 \times V \qquad (3)$$

Table 21: Solute descriptors according to the Abraham model[56] and resulting $logK_{fs}$ values used for the exemplary calculation of transferred analyte amounts in the main paper

Compound name	Reference	Solute descriptors					Resulting
		E	S	A	B	V	$logK_{fs}$
1,2-Dichloroethene (E)	[79,80]	0.425	0.41	0.09	0.05	2.278	2.25
1,1-Dichloroethane	[56]	0.42	0.64	0.1	0.11	0.6352	1.24
1,2-Dichloroethene	[79,80]	0.436	0.61	0.11	0.05	2.439	2.45
2,2-Dichloropropane	[56]	0.37	0.63	0	0.17	0.7761	1.77
Chloroform	[56]	0.43	0.49	0.15	0.02	0.6167	1.66
Carbon Tetrachloride	[56]	0.46	0.38	0	0	0.7391	2.77
1,1,1-Trichloroethane	[56]	0.37	0.41	0	0.09	0.7576	2.36
Benzene	[79,80]	0.61	0.52	0	0.14	2.786	2.61
1,2-Dichloroethane	[79,80]	0.416	0.64	0.1	0.11	2.573	2.56
Trichloroethylene	[56]	0.524	0.37	0.08	0.03	0.7146	2.37
Dibromomethane	[79,80]	0.714	0.67	0.1	0.1	2.886	2.81
Toluene	[79,80]	0.601	0.52	0	0.14	3.325	3.06
Tetrachloroethylene	[79,80]	0.639	0.44	0	0	3.584	3.25
1,1,2-Trichloroethane	[79,80]	0.499	0.68	0.13	0.08	3.29	3.19
1,3-Dichloropropane	[79,80]	0.408	0.74	0	0.17	3.101	2.93
Ethylbenzene	[56]	0.613	0.51	0	0.15	0.9982	3.01
1,1,1,2-Tetrachloroethane	[79,80]	0.542	0.63	0.1	0.08	3.641	3.45
m-Xylene	[79,80]	0.623	0.52	0	0.16	3.839	3.49
o-Xylene	[56]	0.663	0.56	0	0.16	0.9982	2.91
Styrene	[79,80]	0.849	0.65	0	0.16	3.856	3.53
n-Propylbenzene	[56]	0.623	0.5	0	0.15	1.1391	3.59
4-Chlorotoluene	[79,80]	0.705	0.67	0	0.07	4.205	3.82
1,2,3-Trimethylbenzene	[79,80]	0.728	0.61	0	0.19	4.565	4.12
sec-Butylbenzene	[79,80]	0.603	0.48	0	0.16	4.506	4.05
1,2,3-Trimethylbenzene	[79,80]	0.649	0.52	0	0.19	4.344	3.92
p-Isopropyltoluene	[79,80]	0.607	0.49	0	0.19	4.59	4.12
1,3-Dichlorobenzene	[56]	0.847	0.73	0	0.02	0.9612	3.24
1,4-Dichlorobenzene	[79,80]	0.825	0.75	0	0.02	4.435	4.02
n-Butylbenzene	[79,80]	0.6	0.51	0	0.15	4.73	4.24
1,2-Dichlorobenzene	[79,80]	0.872	0.78	0	0.04	4.518	4.09
1,2,4-Trichlorobenzene	[79,80]	0.98	0.81	0	0	5.248	4.71
Naphthalene	[79,80]	1.34	0.92	0	0.2	5.161	4.66

Using these results alongside phase ratios and temperature, it is possible to calculate the extracted analyte amounts by each technique using equation (4)[12], with n_f being the extracted analyte amount, c_0 being the initial analyte concentration inside the liquid sample, K and K_2 as partition coefficients of the analyte between sorption phase and headspace as well as between headspace and liquid sample respectively and V_1,V_2 and V_3 as the volumes of the sorption phase, liquid sample and headspace respectively.

$$n_f = \frac{c_0 \times V_1 \times V_2 \times K}{K \times V_1 + K_2 \times V_3 + V_2} \quad (4)$$

Figure 37 shows an exemplary chromatogram demonstrating sufficient separation of target compounds with the selected chromatographic conditions. Individual compounds may be identified by their retention times given in the main paper.

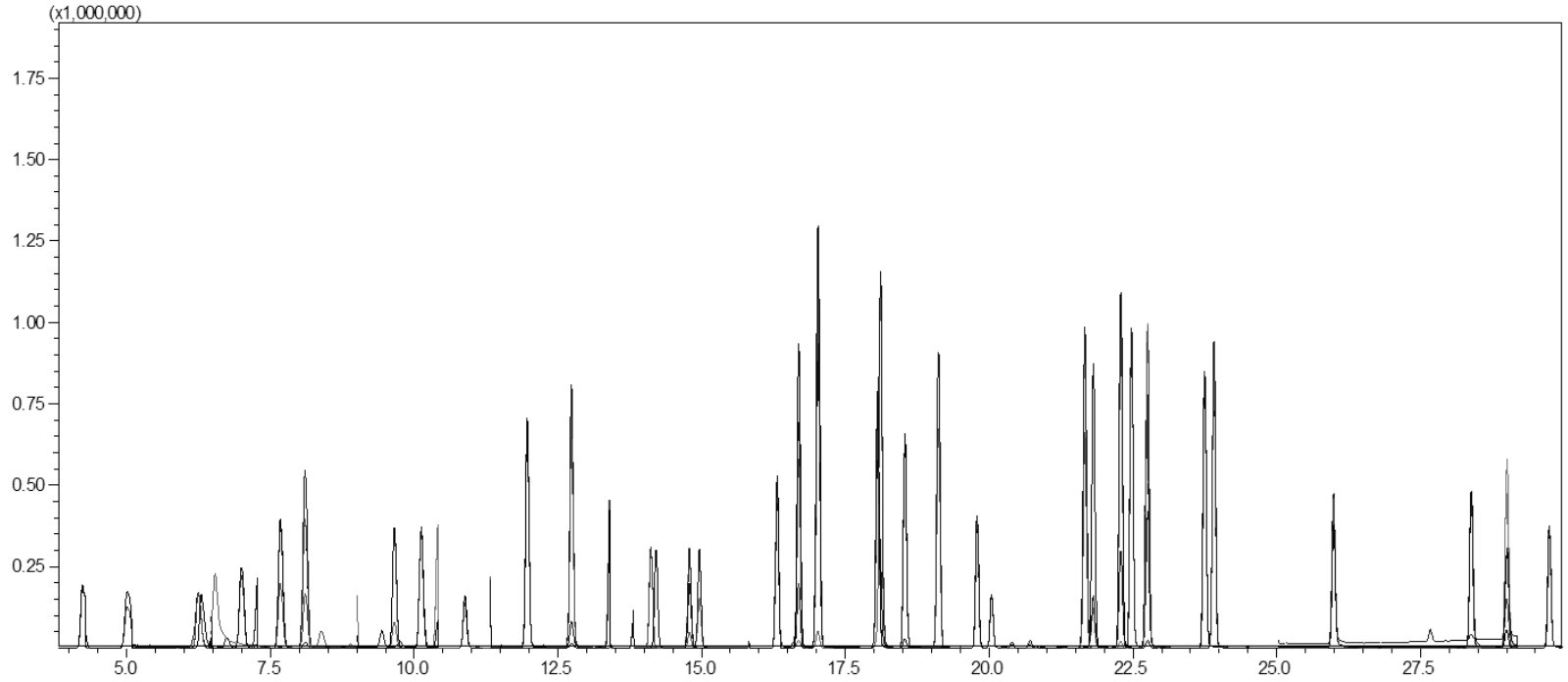

Figure 37: Chromatogram demonstrating sufficient separation of the target compounds used for the comparison of several headspace techniques

Extraction yields. Table 22 contains detailed results of the depletion measurements according to Zimmermann[58] for a selected set of 14 exemplary compounds. Note that this method cannot be applied to techniques that discard large portions of the sample headspace such as the HS-20 Loop.

In case of the latter system, aliquot withdrawal is conducted by venting the pressurized headspace through the loop and ultimately into the vent. Therefore, a larger portion of the analytes is abstracted from the sample vial than is actually transferred into the injector. Since the depletion method calculation is based on declining results with each consecutive analysis of the same sample, a closed system in terms of the sample vessel is indispensable.

The syringe method is different in this matter, since the sample gas inside the syringe is also able to equilibrate with the ambient lab air, but the sample vial remains closed by its septum after sample withdrawal.

Therefore the total loss of analytes in the latter case remains restricted to the calculated 16.5% that exit the syringe during transfer, while the loop system additionally loses headspace gas for filling its internal capillaries and valves (not only the sample loop), as well as due to the pressure relieve by the final vent valve, which is the driving force behind the filling of the loop. The used method of determination is based on performing depletion extractions by extracting and measuring samples multiple times. The declining, logarithmical peak areas are then plotted against the number of consecutive extractions, yielding a linear regression, whose slope then enables calculation of the extraction ratio E from $\log(1-E)$[58] which corresponds to the individual extraction yields shown in Table 22. Figure 38 contains an exemplary plot of such data for a PAL SPME Arrow with a 100 µm 30 mm PDMS sorption phase:

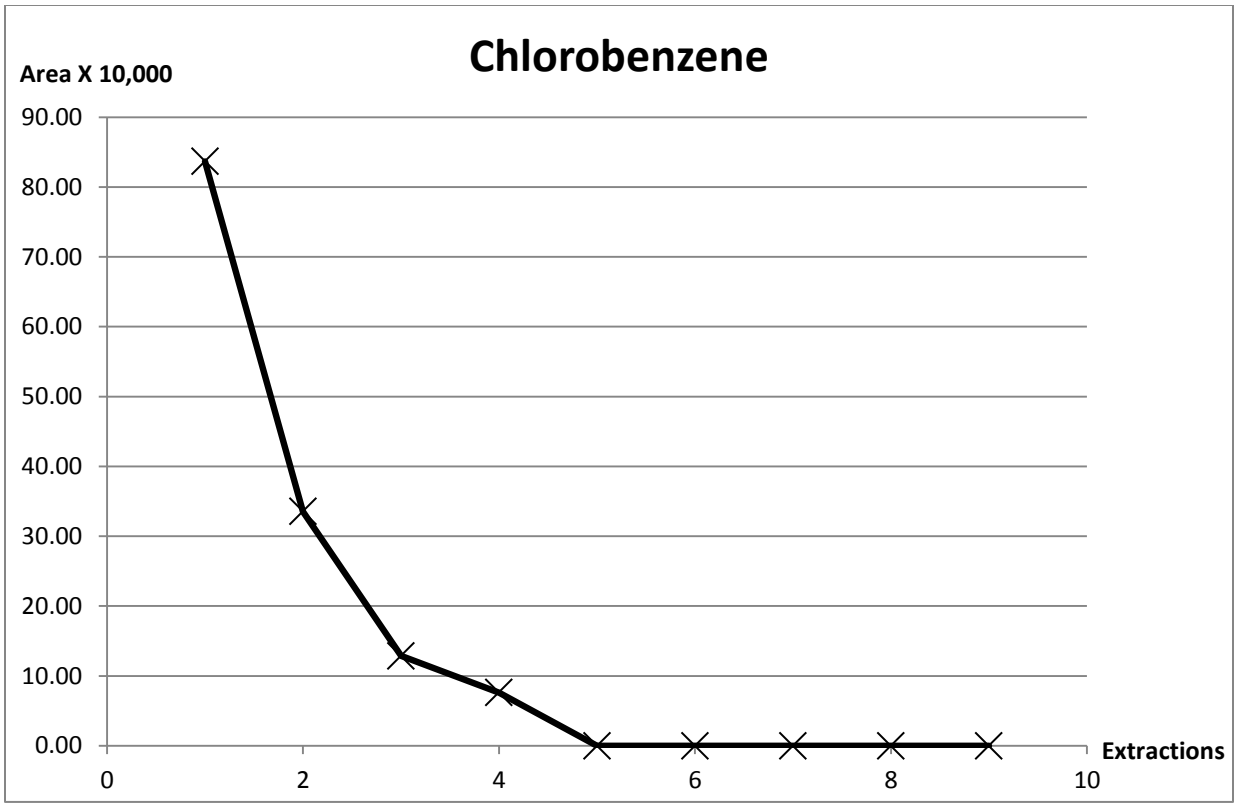

Figure 38: Exemplary plot of data generated for the determination of the extraction yields. Shown data was achieved with a PAL SPME Arrow with a 100 µm 30 mm PDMS sorption phase resulting in a slope of -0.38 and an extraction yield of 58%

Table 22: Extraction yields[58] (%) for exemplary compounds and each individual Headspace technique, experimentally determined for an initial analyte concentration of 1 µg L^{-1}

Compound name	Method name (sorption phase material if applicable) and extraction yields (%)				
	Syringe	ITEX DHS (Tenax TA)	HS-20 Trap (Tenax TA)	SPME (0.6 µL PDMS)	PAL SPME Arrow (10.2 µL PDMS)
Trichloroethylene	10	66	51	6	23
Dibromomethane	3	49	62	6	23
Chlorobenzene	13	70	66	27	58
Ethylbenzene	15	61	74	20	55
1,2-Dimethylbenzene	16	55	71	21	53
n-Propylbenzene	18	78	74	21	55
Bromobenzene	15	60	65	18	50
sec-Butylbenzene	21	83	74	26	66
1,3-Dichlorobenzene	20	50	71	23	55
p-Isopropyltoluene	24	48	67	13	11
1,2-Dichlorobenzene	18	54	65	24	57
n-Butylbenzene	25	50	67	16	35
1,2,3-Trichlorobenzene	20	44	69	43	51
1,2,4-Trichlorobenzene	21	48	65	42	54
Mean over analytes	17	58	67	22	46

Alternative sorption phase materials. Following tables 23 and 24 include the data of additional measurements which were carried out with a reduced set of exemplary analytes in order to evaluate the effects of two different sorption phase materials for PAL SPME Arrow.

Table 23: Measurement data for PAL SPME Arrow equipped with a CTC Analytics 100 µm 20 mm polyacrylate sorption phase used for the extraction of exemplary VOCs from water for the comparison of several headspace techniques

Compound name	Linear Range (ng L^{-1})	Correlation coefficient	MDL [ng L^{-1}] (99% CFI)	Extraction yield (%) (1 µg L^{-1})	RSD 10 ng L^{-1}	RSD 100 ng L^{-1}	RSD 1,000 ng L^{-1}	RSD 10,000 ng L^{-1}
Trichloroethylene	10-10,000	0.99980	2.40	22	4.54	4.77	4.01	4.24
Dibromomethane	10-10,000	0.99989	18.43	9	23.42	6.53	6.59	6.41
Chlorobenzene	1-10,000	0.99967	26.12	25	6.39	7.09	7.50	7.28
Ethylbenzene	1-10,000	0.99947	33.55	27	7.03	7.20	7.96	7.78
1,3-Dimethylbenzene	10-10,000	0.99921	13.80	16	15.12	7.04	7.21	7.90
n-Propylbenzene	1-10,000	0.99896	31.26	29	6.14	6.89	6.66	6.62
Bromobenzene	1-10,000	0.99878	45.61	36	6.43	5.93	5.89	7.11
sec-Butylbenzene	10-10,000	0.99872	5.14	32	6.13	8.17	6.82	6.25
1,3-Dichlorobenzene	1-10,000	0.99769	35.01	42	3.95	6.11	5.84	5.61
p-Isopropyltoluene	1-10,000	0.99445	105.28	41	8.79	7.46	5.81	6.06
1,2-Dichlorobenzene	100-10,000	0.99742	68.13	34	/	6.19	5.52	4.51
n-Butylbenzene	1-10,000	0.99756	40.44	26	5.24	6.21	5.87	4.16
1,2,4-Trichlorobenzene	1-10,000	0.99596	48.19	36	1.43	6.92	7.08	5.02
1,2,3-Trichlorobenzene	10-10,000	0.99394	1.13	42	0.89	6.06	4.86	4.74

Table 24: Measurement data for PAL SPME Arrow equipped with a CTC Analytics 250 µm 30 mm Carbo WR sorption phase used for the extraction of exemplary VOCs from water for the comparison of several headspace techniques

Compound name	Linear Range (ng L^{-1})	Correlation coefficient	MDL [ng L^{-1}] (99% CFI)	Extraction yield (%) (1 µg L^{-1})	RSD 10 ng L^{-1}	RSD 100 ng L^{-1}	RSD 1,000 ng L^{-1}	RSD 10,000 ng L^{-1}
Trichloroethylene	1-10,000	0.99995	8.38	81	16.36	6.18	23.92	11.53
Dibromomethane	10-10,000	0.99966	4.60	77	6.31	4.29	16.30	10.47
Chlorobenzene	1-10,000	0.99988	6.42	75	12.30	6.60	21.78	12.99
Ethylbenzene	1-10,000	0.99940	10.39	55	14.29	7.43	23.41	12.22
1,3-Dimethylbenzene	1-10,000	0.99801	10.97	48	10.24	8.00	21.91	12.11
n-Propylbenzene	1-10,000	0.99828	19.55	47	20.76	6.94	19.68	12.71
Bromobenzene	1-10,000	0.99854	8.35	54	8.79	6.03	16.22	12.90
sec-Butylbenzene	1-10,000	0.99214	0.58	96	1.10	5.19	19.49	11.51
1,3-Dichlorobenzene	1-10,000	0.99719	9.40	44	13.46	6.70	13.94	11.38
p-Isopropyltoluene	1-10,000	0.98971	17.08	81	7.20	5.69	15.48	10.35
1,2-Dichlorobenzene	1-10,000	0.99591	9.55	47	12.46	5.99	13.23	11.05
n-Butylbenzene	10-10,000	0.99576	13.71	60	14.86	6.98	14.57	10.80
1,2,4-Trichlorobenzene	1-10,000	0.99852	8.49	38	12.67	10.54	7.45	10.93
1,2,3-Trichlorobenzene	1-10,000	0.99778	8.36	40	11.43	9.37	8.00	10.88

5 Fully automated SPE with Evaporation and Derivatization for GC/MS Analysis of Phenols in Water

5.1 Introduction

Phenolic compounds are an important class of naturally and anthropogenically produced substances. In organisms they are, e.g., involved in nutrient cycling and bioregulation[95]. For the industry they are important in a variety of applications such as plastics production.

An example for this latter implementation that recently gained critical attention is bisphenol A (BPA). This compound was used in huge amounts for polycarbonate production for, e.g., drinking bottles. Recent studies however demonstrated hormone-like properties of the compound[96], that ultimately resulted in a ban of its use for the production of baby bottles. Also drinking bottles and plastic products that come into direct contact with foodstuffs are now often advertised as being free of BPA.

Contrary to such man-made phenols, there are also naturally occurring examples such as cresols, which can be extracted from coal-tar and which are used in industrial production of polymers and pharmaceuticals[97].

Some phenols and structurally related compounds such as tannins or vanillin are also of sensory importance and contribute to the unique taste of, e.g., special beverages such as wine and whisky[98,99]. Others such as butylated hydroxytoluene (BHT) are used as additives of foods, pharmaceuticals, cosmetics and industrial, e.g., rubber products because of their beneficial - in this case antioxidant properties[100]. BHT can thereby be produced from precursors such as the aforementioned cresols[97].

Halogenated phenols on the other hand possess rather aggressive properties and typically occur as man-made disinfectants, pesticides, herbicides, fungicides, antiseptics and as active ingredients of anti-fouling paints[101].

Due to their persistence in the environment and their described applications, e.g., surface runoff leads to their discharge into aquatic ecosystems.

Some of these compounds such as pentachlorophenol are therefore almost ubiquitous in the environment today[6] and cause notable concern due to their toxicological - and probably carcinogenic - potential[101]. Deemed priority pollutants by the U.S. EPA[102], especially the chlorinated phenols are therefore monitored and regulated. For the same reasons, the European Union regulates the most prominent example from this group of compounds, which is the already mentioned pentachlorophenol[103].

For the analysis of phenolic compounds, two different methods of sample preparation have gained the largest acceptance so far. The first and most straightforward approach is LLE that has also been demonstrated to properly handle more complicated sample matrices such as human plasma. Should subsequent sample preparation steps require the absence of water, drying of the collected organic fractions with, e.g., magnesium sulfate is thereby common [104].

As another common option, SPE is an alternative that is rapid, sensitive and straightforward in its use[105]. While the sample liquid is passing through a sorbent bed, a distribution of analytes between the liquid and stationary phase occurs. The degree of analyte retention - and also the extraction yield - is therefore directly related to the analytes affinity towards the stationary phase[106] and the mass of the latter.

In a more recent approach, the extraction of certain phenolic compounds is also possible via HS-SPME after in situ acetylation in water. This possibility does not only eliminate the notable solvent requirements of the previously described techniques, but also enables a certain degree of analyte selectivity by proper fiber selection[6].

While GC separation is a common choice for the subsequent separation of phenols, their relatively high polarity may lead to problems such as peak broadening and tailing. This can be overcome by derivatization reactions, of which silylation is the most common choice resulting in a decreased analyte polarity and thus improved chromatographic properties[6,105]. Detection can be carried out via electron capture detection (ECD) due to the relatively high electron affinity of especially the halogenated phenols[107] or by universal mass spectrometric detection[106].

Official standards on the topic of phenols differentiate between five methods that are especially suitable for sub-groups of phenolic substances: Phenols and cresols are determined according to DIN 38407-27 via derivatization with subsequent LLE. The, e.g., groundwater samples are thereby consecutively mixed with ascorbic acid, sodium bicarbonate and acetic anhydride. The derivatized analytes are then extracted via n-hexane or cyclohexane and measured via GC/MS after drying with sodium sulfate. The repeatability of the method for drinking water and groundwater in terms of the relative standard deviation (RSD) has been reported to be in the range of 3.3 to 15.4%, while typically detected analyte concentrations are in the range of 1.2 to 5 µg L^{-1}[108].

Very similar to the latter procedure, the European standard DIN EN 12673 recommends derivatization by acetylation for chlorophenols as well. It however adds a simple pretreatment step during which the pH value of the aqueous sample is adjusted to approx. 7 by sodium hydroxide or phosphoric acid. During this method, the derivatization is carried out with a combination of acetic anhydride with potassium carbonate. Extraction of analytes is afterwards done using 100 mL of toluene. Measurement of the finalized samples is again feasible via GC/MS or also GC/ECD (electron capture detector). Typical MDLs in this example were in a range between 0.1 and 27.5 µg L^{-1} and RSDs were between 5 and 22%[109].

For nitrophenols, DIN EN 17495 recommends SPE extraction with, e.g., a polystyrene-divinylbenzene polymeric phase. SPE cartridges are preconditioned with ethyl acetate followed by methanol and acidified water (pH=2). The aqueous samples are as well acidified to a pH < 2 by hydrochloric acid. After enrichment of these samples on the cartridges, the latter are completely dried by a flow of nitrogen until all residual water has been expelled (can take up to 1 h). After elution with ethyl acetate, the analytes are derivatized with diazomethane prior to measurement via GC/MS. Typical analyte concentrations in this case are given as 0.5 to 8 µg L^{-1}, while RSDs are in the range of 7.4 to 20.6%[110]

Similarly, DIN EN ISO 18857-2 contains an SPE method for the analysis of selected alkylphenols, their ethoxylates and BPA. In this case, acetone is used as preconditioning and elution solvent, as well as during derivatization of the analytes with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA). Prior to derivatization, eluates are evaporated to dryness before they are re-dissolved in the solvent in order to eliminate remaining traces of water.

Detected analyte amounts for this method are lower than for the previously described methods (53.7 to 306.3 ng L^{-1}) and RSDs are better as well (2 to 7.8%) when this method is applied to surface water[111].

For the selected alkylphenols 4-nonylphenol and 4-(1,1,3,3-tetramethylbutyl)phenol, DIN EN ISO 18857-1 offers an LLE procedure using toluene to extract the analytes from aqueous samples. After drying with sodium sulfate and enrichment by evaporation, measurement is again conducted via GC/MS. Reported detectable analyte amounts in this case are comparable to the previous examples (0.02 to 2.02 µg L^{-1}) and measurement repeatability in terms of RSDs was in the range of 5.4 to 14.2%[112].

Common properties of these classical methods are the usage of significant amounts of organic solvents (e.g. 100 mL per sample in case of DIN EN 12673) and the dependence on manual workflows. Both contribute to the limited environmental and monetary efficiency of such methods, which could be addressed, e.g., by using miniaturized and automated alternative techniques.

Instrument-top sample preparation (ITSP) cartridges are a novel possibility for full automation of SPE processes via a PAL-type sampler. Preconditioning, loading, transfer and elution of cartridges can be performed automatically, in addition to almost any further sample preparation steps.

Required solvent volumes are thereby significantly reduced by a factor of approx. 10 due to the overall downsizing of these cartridges. Therefore, they could be considered a micro-SPE or MSPE. This option may very well complement other microextraction solutions. While, e.g., the immersion of a SPME fiber into contaminated samples may create problems such as fiber degradation or carryover, an option like ITSP with disposable, constantly renewed sorption phases may prove beneficial.

So far, this new option was used for LC-MS methods. In one work, vitamins were determined in human serum and plasma and ITSP cartridges were used after precipitation and centrifugation, in order to carry out an automated SPE enrichment of the target compounds[35].

In another study, an application was evaluated for the determination of the nicotine metabolite cotinine in biological fluids. Again, an automated ITSP SPE workflow was used in the isolation of the analyte.

The method succeeded in measuring cotinine in human plasma with an MDL of 0.13 µg L^{-1}. It was found especially useful for the batch analysis of large sample sets (>500 samples)[113].

5.2 Materials and methods

Reagents and Materials. Optimization and calibration were carried out by using 1 g L^{-1} stock solutions. These were prepared from pure target compounds and internal standards, which were purchased from Sigma-Aldrich (Steinheim, Germany) and are listed in Table 25. As solvent for these stock solutions as well as for preparation of dilutions and for the washing of syringes, ethyl acetate p.a. also from Sigma-Aldrich was used.

Table 25: Constituents of the prepared phenol stock solutions for evaluation of the ITSP method

Order of elution	Compound name	CAS-Nr.
1	Phenol d5	4165-62-2
2	Phenol	108-95-2
3	4-Methylphenol	106-44-5
4	2-Chlorophenol d4	93951-73-6
5	2-Chlorophenol	95-57-8
6	3-Chlorophenol	108-43-0
7	4-Chlorophenol	106-48-9
8	2-Bromphenol	95-56-7
9	4-Chloro-3-methylphenol	59-50-7
10	2,4-Dichlorophenol	120-83-2
11	2-Nitrophenol	88-75-5
12	Butylated hydroxytoluene	128-37-0
13	4-Chloro-2-methoxyphenol	16766-30-6
14	2,4,5-Trichlorophenol	95-95-4
15	2,3,5-Trichlorophenol	933-78-8
16	2,3,6 Trichlorophenol	933-75-5
17	2,4,6 Trichlorophenol	88-06-2
18	3,4,5 Trichlorophenol	609-19-8
19	Pentachlorophenol	87-86-5

Solid phase extraction was carried out on 10-UDBXP-T ITSP cartridges (shown on the right hand side of Figure 39) obtained from ITSP solutions (Hartwell, USA). As sorption phase, these cartridges contained 10 mg of STYRE SCREEN™ SSDBX material (UCT, Bristol, USA). According to the manufacturer, this material is based on a specially clean, highly cross-linked styrene and divinylbenzene backbone, that is functionalized with benzenesulfonic acid and C18 functional groups. Analytes are retained by a mixed mode interaction consisting of hydrophobic interactions and cation exchange. While this material is not optimal for the enrichment of phenols, the primary target of the initial studies presented here was a general proof of concept for the overall method with a focus on automation.

Figure 39: Three exemplary ITSP MSPE cartridges with different amounts and types of sorption phase materials, as indicated by their distinctive color (10 mg of coconut charcoal for the black and 30 mg of a proprietary QuEChERS blend for the transparent cartridge. Red cartridge was specified above). Sorbent beds are visible in the transparent bottom sections of the cartridges, the upper recessed part serves as a needle guide and the top is outfitted with an 8 mm aluminum crimp cap including a rubber septum

Aqueous samples were prepared in 1-L Schott-flasks using Hamilton glass syringes (Hamilton, Bonaduz, Switzerland), Blaubrand® bulb pipettes (Brand, Wertheim, Germany), lab water from a PURELAB Ultra analytic water purification system (ELGA LabWater, Celle, Germany) and PTFE-coated stir bars. The water was acidified to a pH of 2 using hydrochloric acid (37%, VWR International, Darmstadt, Germany). Calculated amounts, e.g., 1 mL in order to create a concentration of 1 mg L^{-1}, of the previously prepared stock solutions and dilutions were then added and the contents of each flask were thoroughly mixed by magnetic stirring.

MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) as derivatization reagent was purchased from Restek (Bad Homburg, Germany) in a package that contained 10 flasks with a reagent volume of 1 g. These individually sealed flasks guaranteed sufficient freshness of the reagent throughout these studies. Since MSTFA is highly reactive and even interacts with PTFE/silicone/PTFE sandwich septa once they are pierced, the reagent should be renewed at least on a weekly basis.

Transfer of reagent from the 1 g flasks to 2-mL silylated amber vials with above mentioned septa and magnetic crimp caps (all obtained from BGB Analytik, Boeckten, Switzerland) was carried out by Pasteur pipettes (VWR International, Darmstadt, Germany).

GC/MS instrumentation and parameters. All analyses were carried out on a Shimadzu GCMS-QP2010 Ultra (Shimadzu Deutschland GmbH, Duisburg, Germany). 1 µL of finalized sample solution containing trimethylsilyl (TMS) derivatives of the target phenols was injected into a split/splitless injector, which was set to a temperature of 250°C. The injector was equipped with a Restek (3.5 mm I.D. x 5 o.d. mm x 95 mm length) split liner (BGB Analytik, Boeckten, Switzerland). After a splitless time of 30 sec, the split ratio was set to 10:1.

Analyte separation was accomplished on a 30 m x 0.25 mm Rxi®-5ms column (Restek, Bellefonte, PA) with a 0.25 µm film thickness. As carrier gas, Helium 5.0 (Air Liquide, Oberhausen, Germany) with a flow of 1.5 mL min^{-1} was used. The column oven temperature started at 50°C which was maintained for 2 min before increasing at a rate of 2°C/min to 130°C. After this a second ramp at 15°C/min was carried out until 280°C which was held for 8 mins resulting in an overall GC runtime of 60 min.

The transfer line and ion source were both set to 250°C, respectively. Retention times varied between 14 and 51 min for phenol-TMS and pentachlorophenol-TMS respectively with sufficient separation of peaks as demonstrated in Figure 40:

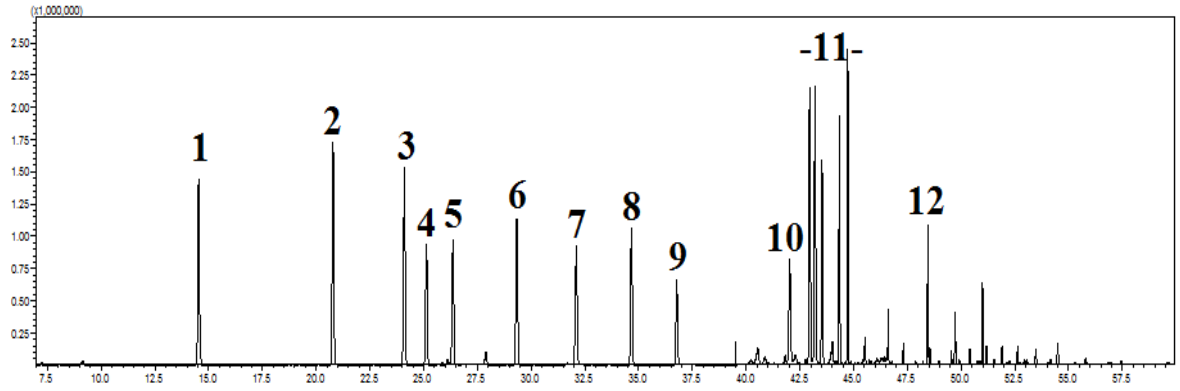

Figure 40: TMS-derivatives of analyzed phenols, extracted and derivatized via the developed, automated method, measured by GCMS: (1) phenol, (2) 4-methylphenol, (3) 2-chlorophenol, (4) 3-chlorophenol, (5) 4-chlorophenol, (6) 2-bromophenol, (7) 4-chloro-3-methylphenol, (8) 2,4-dichlorophenol, (9) 2-nitrophenol, (10) butylated hydroxytoluene, (11) 4-chloro-2-methoxyphenol, 2,4,5-trichlorophenol, 2,3,5-trichlorophenol, 2,3,6 trichlorophenol, 2,4,6 trichlorophenol and 3,4,5 trichlorophenol , (12) pentachlorophenol.

Mass spectrometry. The mass spectrometer was regularly tuned with FC43 (perfluorotributylamine, CAS 311-89-7) (BGB Analytik, Boeckten, Switzerland). Ionization of analytes was performed via electron impact ionization (ionization voltage = 70 V, emission current = 150 µA, temperature = 250°C) and initial identification of the TMS derivatives of the phenols was carried out in total ion current (TIC) mode (detector voltage = 0.96 kV) in a specified m/z range of 50 to 350. Quantification of the phenols was then conducted in selected ion monitoring (SIM) mode, using the specific m/z ratios of the individual analytes shown in Table 26. To reduce the number of simultaneously monitored m/z ratios, 11 different SIM segments were used. In addition, the identification of the analytes was assured by monitoring two specific reference ions at the corresponding molecules' characteristic retention times. Results for phenol-TMS and 4-methylphenol-TMS were normalized via phenol-d_5-TMS, while all other compounds' results were normalized via the second internal standard 2-chlorophenol-d_4-TMS.

Table 26: Quantifier and qualifier ions for the investigated phenolic compounds as TMS derivatives that were used as exemplary analytes for the evaluation of the ITSP method

Order of elution	Compound name	Quantifier ion	Qualifier ion
1	Phenol d5	156	171, 157
2	Phenol	151	166, 152
3	4-Methylphenol	165	91, 180
4	2-Chlorophenol d4	93	95, 189
5	2-Chlorophenol	149	93, 185
6	3-Chlorophenol	185	187, 93
7	4-Chlorophenol	185	73, 200
8	2-Bromphenol	149	139, 137
9	4-Chloro-3-methylphenol	199	73, 214
10	2,4-Dichlorophenol	93	219, 95
11	2-Nitrophenol	97	198, 196
12	Butylated hydroxytoluene	253	255, 93
13	4-Chloro-2-methoxyphenol	93	95, 255
14	2,4,5-Trichlorophenol	73	200, 215
15	2,3,5-Trichlorophenol	205	57, 220
16	2,3,6 Trichlorophenol	93.00	253, 255
17	2,4,6 Trichlorophenol	73.00	253, 255
18	3,4,5 Trichlorophenol	93.00	73, 255
19	Pentachlorophenol	93.00	73, 323

PAL RTC equipment. While modules are attached to the main beam (X-axis) of the RTC sampler, tools are stored in their respective park stations. The latter are picked up by the arm of the sampler (Z-axis) and carry, e.g., syringes for interacting with various liquids.

Seven different tools were used in the phenol sample preparation sequence. They are listed in Table 27 alongside the syringes or needles that they were outfitted with. Prior to all liquid handling steps, syringes were subjected to a sequence of three filling strokes at 30% of their total capacity for avoidance of air bubbles. Prior to the transfer of derivatization reagent and final sample injection, the corresponding syringes were in addition subjected to a rinsing procedure, during which 5 µL of reagent or sample were transferred to the corresponding waste ports in order to remove residual wash solvents from these syringes almost entirely.

This avoids, e.g., dilution of the aspired 1 µL of final sample with solvent that remained inside the syringe from the previous washing steps. No filling strokes were performed prior to such rinsing procedures in order to avoid dilution of the derivatization reagent and final samples. As with all parameters contained in the sampling cycle, transferred amounts, penetration depths, withdraw- and dispense speeds, stroke amounts and waiting times could be freely adjusted during the washing and rinsing procedures.

Table 27: Tools used during PAL RTC sequence for automated SPE, derivatization and injection of phenols from water. The MHE abbreviation is synonymous for multiple headspace extraction

Nr.	Designation	Type	Syringe / Needle	Use
1	D7/57	Liquid	10 µL	Sample injection
2	D7/57	Liquid	100 µL	Cartridge conditioning
3	D7/57	Liquid	100 µL	Reagent transfer
4	D8/57	Liquid	1000 µL	Elution & transfer
5	HS2500	Headspace	2500 µL	Eluate evaporation
6	Prototype	Dilutor	Gauge 23 needle	Sample loading
7	Prototype	MHE	Fixed needle	Eluate evaporation

The modules that were attached to the PAL RTC and used in this method are given in Table 28 alongside their function. Note that the waste port of the large volume wash station and the waste receiver below the loading rack of the ITSP cartridges were connected to a 2.5-L Schott-flask that served as a convenient waste receiver for the large sample volumes that were handled by the sampler (Figure 41).

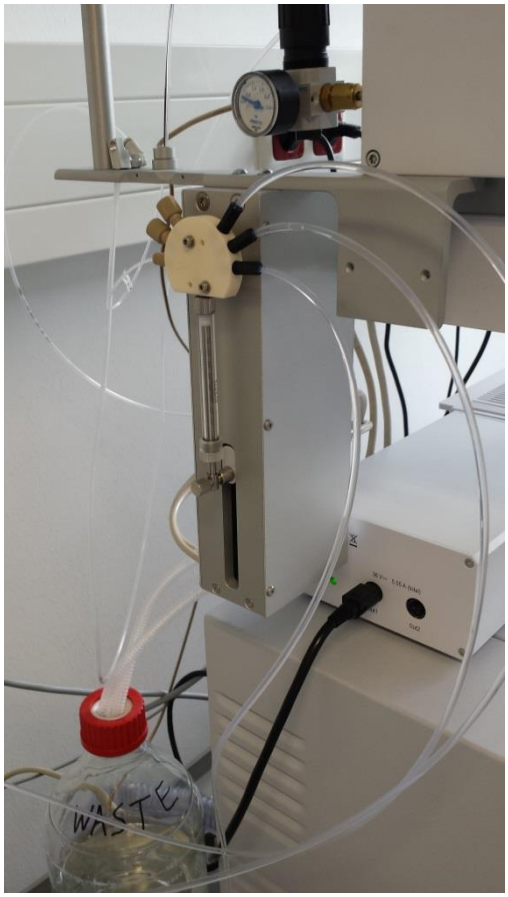

Figure 41: The dilutor module used to supply the PAL RTC sampler with aqueous phenol samples for loading of the ITSP cartridges. The waste bottle for the subsequent collection of the extracted water samples is visible as well

Samples and rinsing solvent were stored in 1-L Schott-flasks and attached to the dilutor module via tubes (Figure 45). Up to 5 of such flasks could be connected to the sampler in parallel. Changing these flasks had to be done manually. The exhaust port of the MHE tool was attached to the vent in order to dispose, e.g., evaporated solvent safely. As an additional, external module, an Aalborg GFC 17 thermal mass flow controller (Aalborg, New York, USA) was employed in order to homogenize the flow of nitrogen into the PAL sampler. An overview over the sampler outfitted for this study is given in Figure 42:

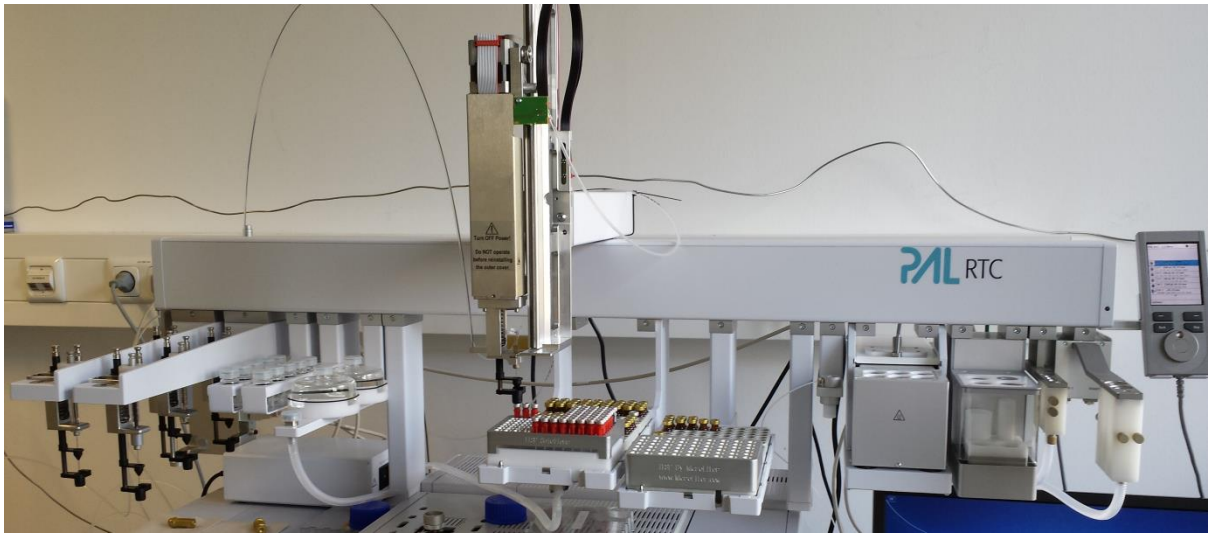

Figure 42: Overview over the PAL RTC sampler as outfitted for the automated SPE, derivatization and injection of phenols from water. Installed modules starting at the left side are: Two park stations for tools, two standard wash modules, one large-volume wash module, one ITSP-compatible vial rack, one standard vial rack, MHE module, agitator, vortex mixer and two fast wash modules not used in these experiments

Table 28: Modules used during PAL RTC sequence for automated SPE, derivatization and injection of phenols from water

Nr.	Designation	Type	Use
1	MkII	Trayholder	Rack mount / ITSP compatible
2	MkI	Trayholder	Rack mount
3	VT54	Rack 2 mL	Eluate processing
4	VT54	Rack 2 mL	Reagent storage
5	VT54	Rack 2 mL	Vial storage
6	VT54	Rack 2 mL	Vial storage
7	VP54	Rack 2 mL	Eluate receiver
8	ITSP96	ITSP Prep	Cartridge loading / Waste receiver
9	VT54ITSP	ITSP Elute	Cartridge elution
10	Wash module	Standard	Wash tool 1
11	Wash module	Standard	Wash tool 3
12	Wash module	Large	Rinse tool 6 / Solvent storage
13	Park station	Standard	Storage of tools 4 - 6
14	Park station	Standard	Storage of tools 1 - 3
15	Park station	MHE	Storage of tool 7
16	Dilutor	Multivalve	Sample supply of tool 7
17	Agitator	Standard	Thermostatting during evaporation
17	Vortex mixer	Standard	Sample mixing

Used vials and caps. During these experiments, 2-mL amber glass vials were used for intermediate storing and further processing of the eluates. (BGB Analytik, Boeckten, Switzerland). Those that were used as eluate receivers (module 7) were outfitted with integrated inserts in order to reduce their internal volume to 300 µL which facilitates the subsequent quantitative withdrawal of liquid.

Especially the choice of caps that can be used alongside ITSP cartridges is not a trivial selection. The unique property which is demanded of vial septa during elution of the cartridges is that they must not seal entirely. Air from inside the vial has to be able to exit it during the elution process or otherwise pressure is building up and resisting the elution process leading to an overflow of the cartridge.

So far, only Restek 8 mm snap caps (Restek, Bad Homburg, Germany) outfitted with starburst® septa proved adequate for this task. Aside these elution processes, crimp caps with PTFE/silicone rubber/PTFE septa were used (also BGB Analytik).

Sequence programming. The program code was developed in PALscript Editor V2.2 Internal-Beta (CTC Analytics AG). This scripting solution for the novel PAL3 platform is completely different from the macro-based workflow of the previous PAL2 (e.g. PAL-xt) generation and resembles classical software coding such as Visual Basic.

An exemplary piece of code controlling cartridge elution is shown in Figure 43 and the overall code had a length of approx. 600 lines. The program code is presented in its entirety in the supporting information to this chapter (5.5).

```
// Elution

SetTemperature( target=agitator, temperature=incubationTemperature, wait=waitForReadi
ChangeTool( tool=syringeForElution)
MoveToObject( target=fillupSolventSource, index=fillupSolventIndex)
PenetrateObject( target=fillupSolventSource, index=fillupSolventIndex, depth=fillupSo
FillingStrokes( volume=fillupSolventFillingStrokesVolume, aspirateFlowRate=fillupSolv
Wait( time=delayAfterFillupSolventFillingStrokes)
AspirateSyringe( volume=elutionSolventVolume, flowRate=fillupSolventAspirateFlowRate)
LeaveObject()
MoveToObject( target=rackITSPCartridge, index=sampleIndex)
PenetrateObject( target=rackITSPCartridge, index=sampleIndex, depth=dilutorCartridgeP
TransportVial( source=cartridge, destination=elutionRack, destinationIndex=sampleInde
EmptySyringe(flowRate=elutionSolventFlowRate)
Wait(time=elutionSolventPostDispenseDelay)
TransportVialHome( vial=cartridge, leaveObject=false)
Depenetrate()
MoveRelative( movementZ=-2mm, forceDirectMovement=true)
MoveRelative( movementY=-5mm, forceDirectMovement=true)
LeaveObject()
```

Figure 43: Excerpt of code for the elution process constituting the automated ITSP SPE procedure on the CTC PAL RTC autosampler

The programmed sequence was then implemented for measurement series using PALscript Executor V2.2 Internal-Beta (CTC Analytics AG) in correlation with a V2.2.4 CTC PAL RTC Firmware build (2.2.4-2.2.15259.1545). Individual execution parameters of the method sequence could thereby either be hardcoded into the programming, pre-adjusted during conversion of the raw code into the .XML sequence file and subsequent loading of the latter file into the script executor, or specifically adjusted for each individual sample during final setup of the sample list.

Method sequence. The evaluated SPE method was developed on the basis of the official standards DIN EN ISO 17495[110] and DIN EN ISO 18857-2[111]. However, it also has to take into account that ITSP is comparable to classical SPE but not fully identical in its possibilities. The absence of vacuum during preconditioning, loading and elution of the cartridges is the most significant example here and will be further discussed in the results section. A schematic of the programmed method sequence is shown in Figure 44. Details concerning the consecutively numbered steps of the process will be given in the following.

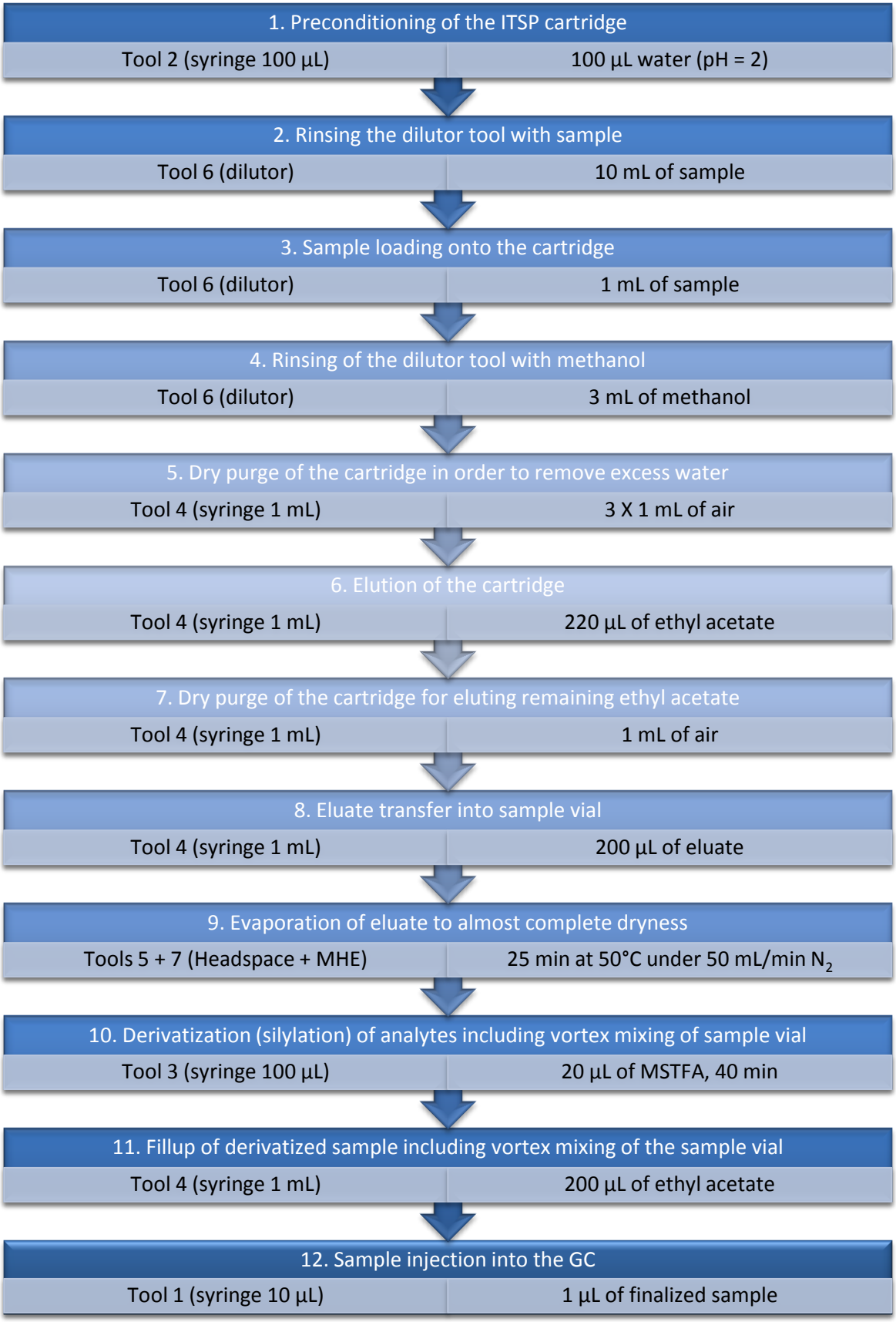

Figure 44: Schematic of the programmed method sequence for automated phenol analysis using ITSP cartridges on a PAL RTC

1. As with all SPE techniques, the first step is a preconditioning of the used cartridges and sorbents in order to ensure purity and proper retention of analytes from the beginning of the loading sequence. Different solvent sequences were evaluated for this purpose such as methanol succeeded by acidified water (pH = 2), ethyl acetate succeeded by acidified water and direct application of acidified water without prior application of an organic solvent. 100 µL of each individual solvent were thereby slowly (flow = 0.5 µL s^{-1}) dispensed through the corresponding cartridges by tool 2 (syringe 100 µL).

2. After preconditioning, tool 6 (dilutor) was mounted by the PAL and rinsed with 10 mL of sample at the waste port of module 12 (large volume wash module) at a flow of 100 µL s^{-1}.

3. Subsequently, the tool was moved to rack 6 and penetrated the preconditioned ITSP cartridge. The desired sample volume (always 1 mL in this study) was then slowly (flow = 0.5 µL s^{-1}) dispensed through the sorbent bed so that the enrichment of analytes could take place. Larger flows were thereby avoided after initial trials showed overflowing cartridges due to backpressure by the narrow sorbent beds (Figure 46).

4. After successful loading of the cartridge, the tool was transferred back to the waste port of the large volume wash module and rinsed with methanol (5 mL) from the attached wash solvent bottle.

5. After rinsing, the dilutor was switched out for tool 4 (1 mL syringe). The latter was first of all used for a simplified drying step of the loaded cartridge. For this it aspired 1 mL of ambient lab air and dispensed it through the cartridge (100 µL s^{-1}).

Clearly visible amounts of water were expelled out of the cartridge by this procedure during the first time and it was repeated two more times for a total of three dry purge steps.

6. Afterwards the elution was started by aspiring 220 µL of ethyl acetate from the large volume wash module. The filled syringe then penetrated into the loaded cartridge and performed a needle transport sequence in order to transfer the cartridge to rack 3, where it was set on top of an empty vial with integrated 300 µL insert. Here, analytes were slowly eluted from the cartridge at a flow of 1 µL s^{-1}. Again, larger flows had to be avoided due to potential overflowing of cartridges as demonstrated in Figure 47.

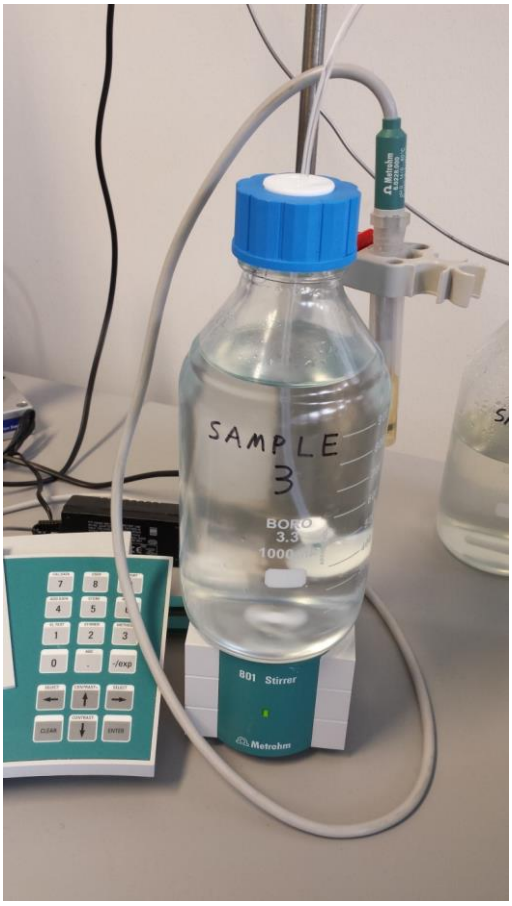

Figure 45: 1-L Schott flask containing a stirred, acidified water sample during extraction. The intake of the dilutor tool is clearly visible, resembling the solvent intakes of HPLC instruments

Figure 46: Overflowing ITSP cartridge due to backpressure during loading with a flow of 5 µL s^{-1}

Figure 47: Overflowing ITSP cartridge due to backpressure during elution with a flow of 5 µL s^{-1}

7. Afterwards, the cartridge was returned to its origin and the full elution cycle (**6.**) including the needle transport was repeated for another time, but with 1 mL of ambient air instead of further solvent. This procedure was similar to the initial dry purge step after cartridge loading and fulfilled a very similar purpose. Without it, there were significant amounts of solvent (and thus analytes) still present in the cartridge when it was discarded. This step was included after initially unsatisfactory standard deviations of measured signals and inconsistent eluate volumes, which could be remediated to a large degree by this additional purge procedure.

8. After completion of the elution cycle, the eluate was then transferred by the same tool to a sample vial on rack 1. The transfer volume was thereby set to 200 µL in order to achieve reproducible results, meaning that 20 µL of eluate were not further processed. The eluate could not be further processed in the elution vial, since this was locked in its position by the overlaying aluminium well plate shown in Figure 47. Furthermore, the here used vials with conical 300 µL insert are suitable for achieving high recovery during liquid transfers, but also possess specific limitations such as a smaller surface of the contained liquids. This limits their usefulness during evaporation procedures.

9. The further sample preparation was commenced by selection of tool 1 (10 µL syringe) for magnetic transport of the sample vial to position 1 of the agitator.

This is not possible with the tools that penetrate and transport the ITSP cartridges, since the latter were outfitted with modified needle guides for an improved centering of the narrow cartridges during penetrations and needle transports.

These modified needle guides are supplied alongside the other specialized ITSP instrumentation and lack the magnets that standard needle guides possess for magnetic vial transfers. While the PAL switched to tool 5 (headspace), the sample had approx. 1 min to equilibrate to the temperature inside the agitator (50°C). After selection of the appropriate tool, the additional MHE tool (Figure 48) was mounted to the needle guide of the headspace tool at the MHE module.

Figure 48: MHE tool used for eluate evaporation during ITSP method evaluation displaying the additional needle with connection to the vent (red circle)

The plunger of the headspace syringe was then brought into the purge position and the nitrogen valve of the PAL sampler was opened. The MFC-controlled flow of nitrogen through the sampler (50 mL min^{-1}) was given 10 sec to equilibrate in order to purge any residual air from the flow lines.

Afterwards this combination of tools was transferred to the agitator and the sample vial was pierced by the syringes of both the headspace and the MHE tool as shown in Figure 49.

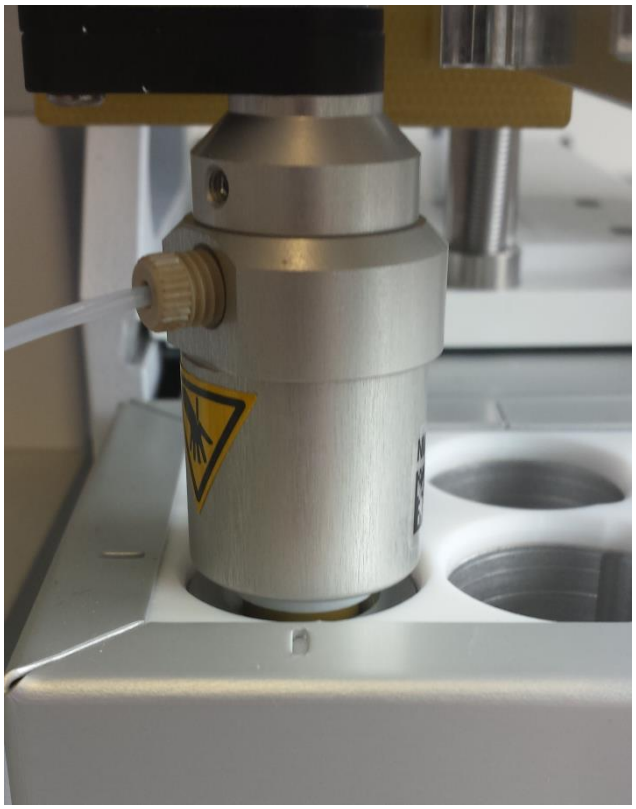

Figure 49: Simultaneous penetration of a vial containing eluate by the HS and MHE tools inside the heated agitator for controlled and safe evaporation of solvent into the vent

Thereby, the nitrogen purge flow flushed through the sample vial, through the syringe of the MHE tool and ultimately into the vent, enabling a safe evaporation of the eluate inside the vial. The evaporation time and temperature were - just like the other parameters of the overall procedure - adjustable, which resulted either in complete evaporation to dryness or a certain degree of residual solvent.

In order to achieve the latter possibility, an evaporation time of 25 min at a temperature of 50°C was chosen after extensive optimization, leading to an almost exhaustive evaporation with only a thin film of solvent left at the bottom of the vial. All remaining traces of water inside the sample vial were removed within this time span. Water otherwise impeded the subsequent derivatization of the analytes, leading to irreproducible peaks for their trimethylsilyl-derivatives.

10. After evaporation, tool 7 (MHE) was returned to module 15 and the eluate vial was also returned to its origin using tool 1. Afterwards, tool 3 (syringe 100 µL) was used in order to add 20 µL of MSTFA to the eluate, which was then transported to the vortex mixer for one minute of efficient mixing at 1200 rpm. Subsequently, the vial was returned to its origin again, where it was left until the derivatization time of 40 min had passed. Figure 50 displays the MHE module, the agitator and the vortex mixer in close up:

Figure 50: MHE module containing the MHE tool, agitator and vortex mixer used in this work

11. The last remaining step after derivatization was then to fill up the sample vial with ethyl acetate in order to have enough volume inside the vial for further withdrawal. Using standard flat-bottom vials, at least 200 µL of liquid have to be present in order to allow for, e.g., 1 µL to be withdrawn reliably for the subsequent injection into the GC injector. This however caused a necessary dilution of the sample. The best case would of course be, if the sample was present in a narrower vial at this point, so that the fill up can be avoided, resulting in better signal intensities during analysis (approx. factor 4). Such narrower vials however proved unsuitable for evaporation of the eluate, probably due to the insufficient surface area of the liquid.

The transfer of the eluate into such narrower vials after derivatization would also require the vial of origin to be filled up in order to withdraw eluate and is therefore not a suitable option.

12. Injection into the gas chromatographic system was carried out by tool 1 (syringe 10 µL). Thereby the novel possibility of fast injection was typically used, since it provides a minimum potential for analyte discrimination due to undesired thermospray effects. This feature was also introduced with the PAL 3 sampler generation and combines the entire injection procedure (injector port penetration, plunger depression, needle retraction) into an extremely quick sub-script that is performed in a mere 100 ms instead of approx. 2 to 3 sec that were required with the older sampler generations.

After injection, the sampler was then ready to start preparation of the next sample. The overall analysis time for a single sample was approx. three hours, depending on the desired sensitivity of the method which influences especially the necessary loading time (corresponding to loaded sample volume - more sample = higher sensitivity).

5.3 Results and discussion

Method parameter optimization. Besides setting up the instrumental and programming basis of the described method, fundamental work was carried out on optimizing and evaluating its analytical potential for the analysis of phenolic compounds from water samples.

The flexibility of the methods programming in combination with the multitude of individual steps during its sequence thereby leads to a considerable amount of approx. 140 adjustable parameters such as sample loading volume, evaporation time and temperature, syringe wash cycles, derivatization reagent volume, length and intensity of vortex mixing, sample injection flow etc.

The generation of initial results was based on selection of the most important parameters, which were varied while the remaining parameters were kept constant at values that appeared meaningful due to literature research and/or personal communication with experts.

Cartridge preconditioning. The first procedure to be optimized was the ITSP cartridge preconditioning. Common SPE methods usually involve a cartridge preconditioning with organic solvents. During the course of this study, however, best results were achieved for a sole preconditioning step with acidified water, so that this variant was used for all further preconditioning sequences. Results are displayed in Table 29 and show a comparison of the RSD that could be obtained by using either only acidified water or a combination of the latter with a prior application of methanol or ethyl acetate.

Table 29: RSD resulting from different solvent combinations used for cartridge preconditioning during ITSP method evaluation

Compound	Only water	Water + methanol	Water + ethyl acetate
Phenol TMS	4.7	11.1	11.6
4-Methylphenol TMS	2.1	6.2	11.6
2-Chlorophenol TMS	5.3	9.7	23.2
3-Chlorophenol TMS	17.5	10.9	3.0
4-Chlorophenol TMS	8.6	10.4	6.4
2-Bromphenol TMS	8.0	10.5	6.9
4-Chloro-3-methylphenol TMS	2.6	10.7	6.0
2,4-Dichlorophenol TMS	2.5	11.2	13.6
2,4,5-Trichlorophenol TMS	4.7	9.5	19.1
2,3,5-Trichlorophenol TMS	5.4	10.7	17.9
4-Chloro-2-methoxyphenol TMS	2.3	10.7	55.5
Butylated hydroxytoluene	21.1	50.5	42.6
2,3,4 Trichlorophenol TMS	10.6	8.9	17.9
2,4,6 Trichlorophenol TMS	7.7	11.4	29.6
3,4,5 Trichlorophenol TMS	2.4	12.0	16.1
Pentachlorophenol TMS	4.5	10.4	16.2
Mean	6.9	12.8	18.6

The unsuitability of a conditioning step involving organic solvent may be connected to the lacking possibility of applying any suction to the cartridges. An additional PAL module could be proposed here, that connects the bottom of the cartridges not only to the waste bottle but in addition also to the vent or to the suction generated by an additional syringe such as the one present in the dilutor module.

However for the time being, reliable removal of residual solvent proved difficult, so that even after rinsing with acidified water, there was possibly still a non-reproducible amount of, e.g., ethyl acetate present inside the cartridge. These residual amounts of solvents may then interfere in the subsequent loading of the cartridges with sample, creating an additional organic phase that transports inconsistent amounts of analytes through the sorbent bed and out of the cartridge.

Solvents and derivatization times. The solvent used for elution, derivatization and injection of the analytes can be expected to have a major impact on the method's sensitivity. Five different solvents were evaluated alongside two different derivatization times. Figure 51 shows that for all derivatizations except for the ones occurring in methanol, a longer timeframe has beneficial effects on the resulting method sensitivity in terms of mean peak areas.

Best results were obtained for ethyl acetate, so that this solvent was used for all subsequent measuring sequences.

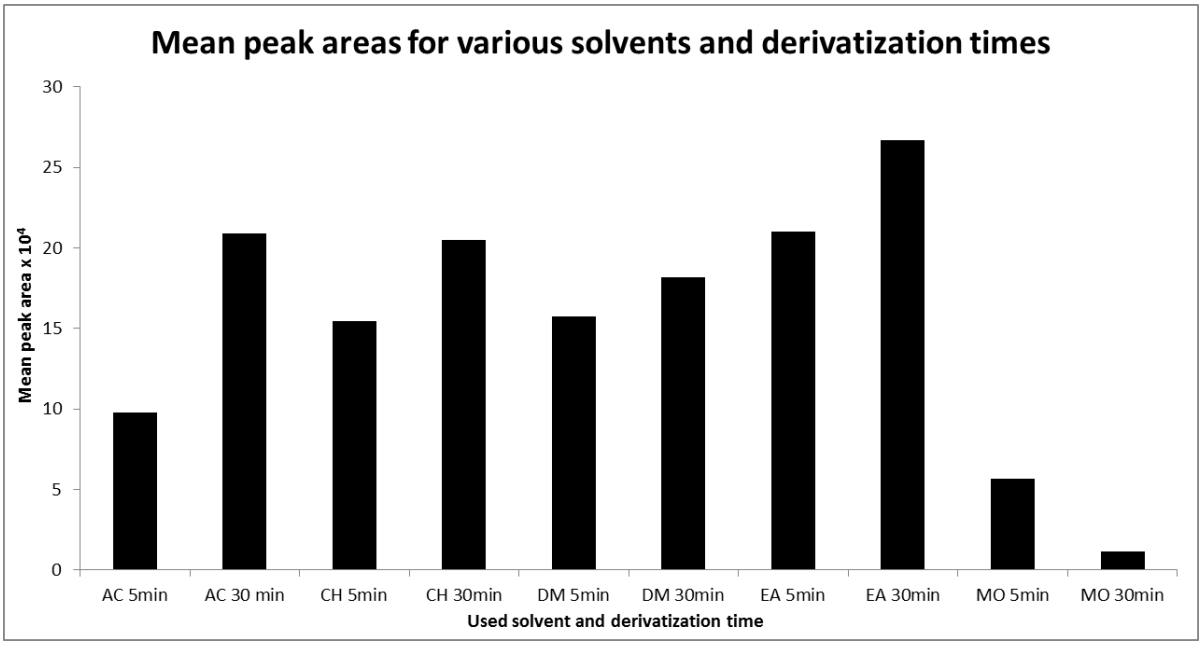

Figure 51: Mean peak areas obtained with various combinations of solvents and derivatization times. AC = acetone, CH = cyclohexane, DM = dichloromethane, EA = ethyl acetate, MO = methanol

Reagent stability. The stability of the used derivatization reagent (MSTFA, obtained from Restek GmbH, Bad Homburg, Germany), was evaluated over the course of 90 hours after the initial piercing at 0 hours. The results are displayed in Figure 52 and show how practically constant results can be obtained within the first approx. 24 hours after the initial opening of the reagent. After 60 hours, a decrease in effectiveness by a factor of 14% was observed. For this reason, measurement series that involved comparison of method sensitivities, e.g., for water removal options were always performed using fresh derivatization reagent, opened at the day of measurement.

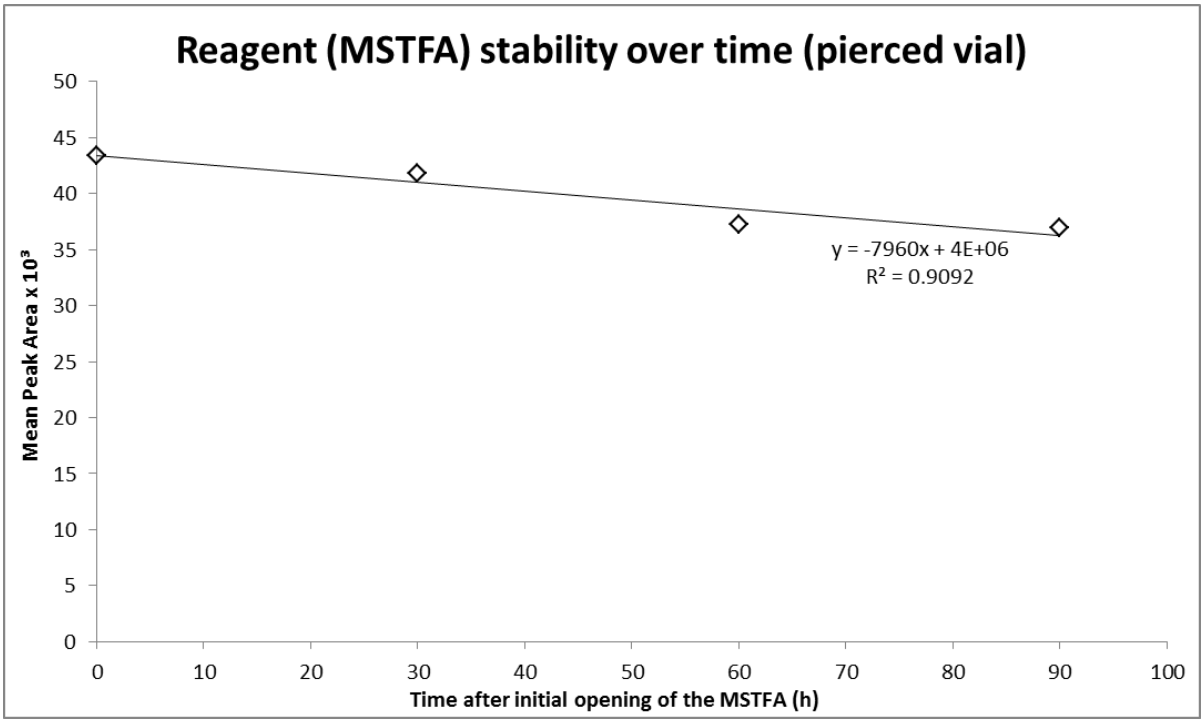

Figure 52: Mean peak areas resulting from repeated measurements over a timeframe of 90 hours using the same vial of derivatization reagent (MSTFA), showing a decrease in effectiveness over time

Impact of residual water. Similar to the residual solvent problems during sample loading, additional issues were monitored with residual water on the cartridges after loading with sample. As shown in literature[106], minimization of such residual water is already difficult if options such as vacuum drying are available. This is not the case with the ITSP solution, so that a certain amount of residual water is unavoidable in this case. This requires the user to implement drying procedures since the subsequent derivatization processes would otherwise be disturbed by the water.

The impact of water on the derivatization process was evaluated by directly preparing phenol samples in ethyl acetate without an extraction from water. Prior to derivatization by MSTFA however, these samples were spiked with defined amounts of water. All other parameters were thereby kept constant. Figures 53 and 54 show, how an increased amount of water in the samples lead to higher RSDs and smaller method sensitivity in terms of mean peak areas. Minimization of residual water was therefore deemed crucial for the further optimization of the automated MSPE method.

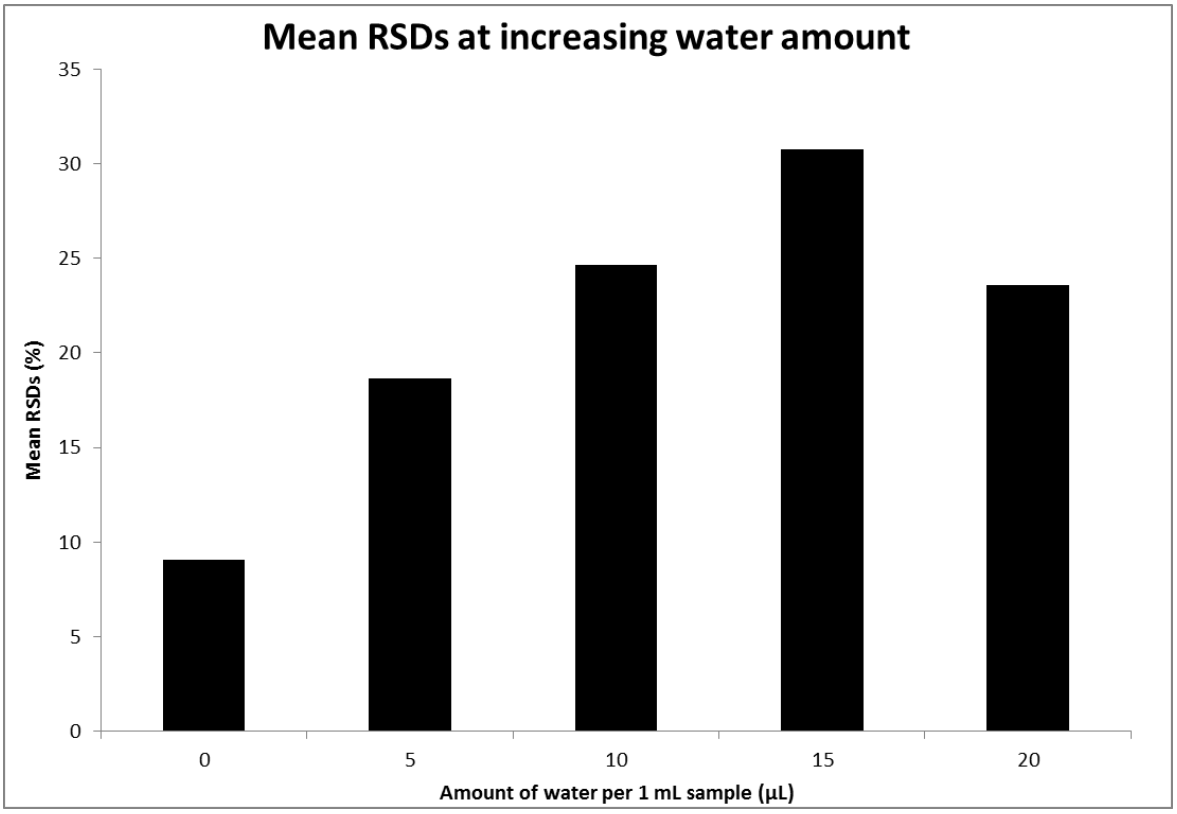

Figure 53: Mean RSDs resulting from increasing amounts of spiked water in phenol samples that were directly prepared in ethyl acetate without prior extraction (each 1 mL) for evaluating the impact of water on the derivatization method

Eluate drying. Prior to using the automated evaporation procedure, it was attempted to dry the eluate chemically with sodium sulfate, magnesium sulfate and molecular sieves with a diameter of 3Å (All obtained from Sigma Aldrich, Steinheim, Germany). All chemical drying steps were initiated by a vortex mixing of the eluate with the drying reagent at 1500 RPM for 1 min. Afterwards the two remained in contact for a defined amount of time (contact time) before the supernatant sample was again transferred into another vial for derivatization.

All three possibilities, shown in the figures 55 to 57, however resulted in a significant decrease in obtained signal intensities. This might have been caused by sorption of phenols to the drying agents and the option of chemical drying was abandoned. Perhaps, similar observations resulted in the official standard recommendation, to carry out the chemical drying subsequent to the derivatization of the phenols[108,109].

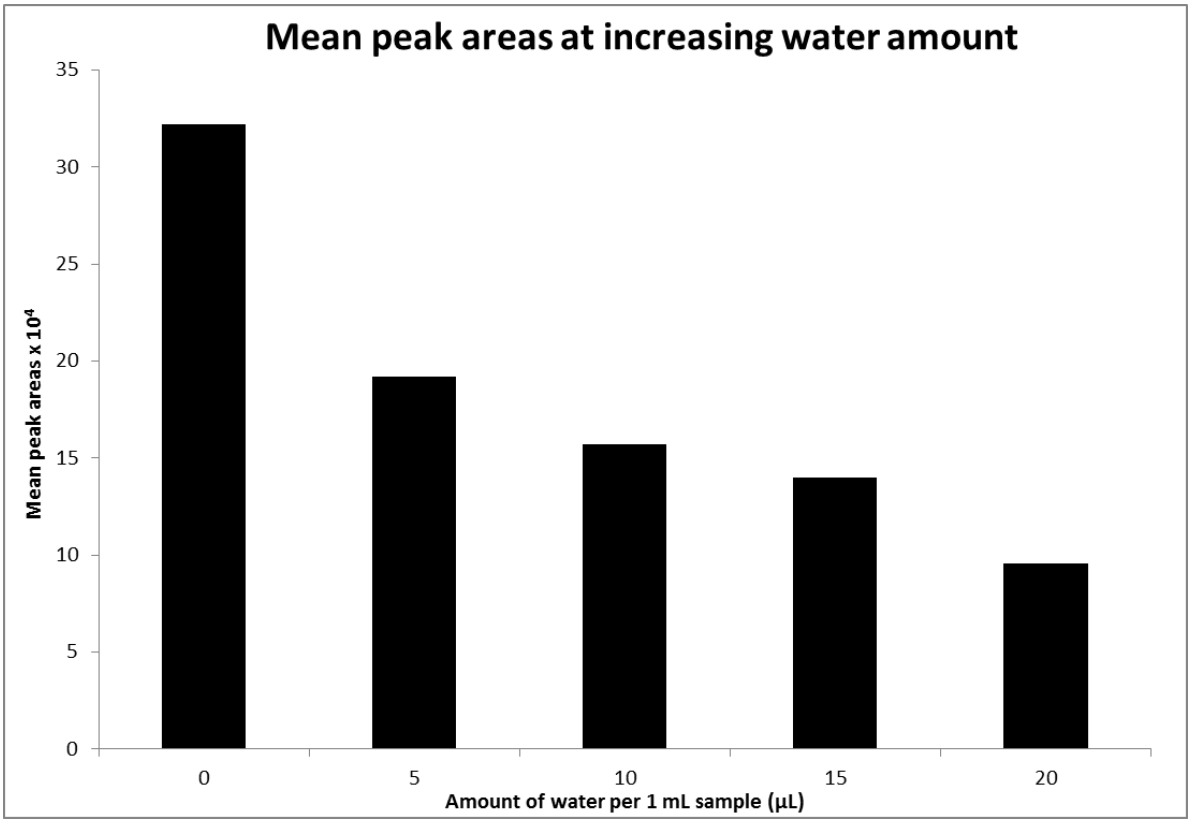

Figure 54: Mean peak areas resulting from increasing amounts of spiked water in phenol samples that were directly prepared in ethyl acetate without prior extraction (each 1 mL) for evaluating the impact of water on the derivatization method

The strongest decrease in signal intensity was found for magnesium sulfate, followed by sodium sulfate. The molecular sieves had the least effect on signal intensity but still showed a significant decrease in that regard, with increasing contact time. The higher affinity of phenols to magnesium in comparison to sodium might be constituted by the larger electronegativity of magnesium.

The use of magnesium sulfate additionally resulted in a significant loss of time, since this option created a well visible cloudiness inside the vials. In order to avoid any potential clogging of the transferring syringe, it would have been inevitable to wait approx. one hour for this turbidity to settle completely.

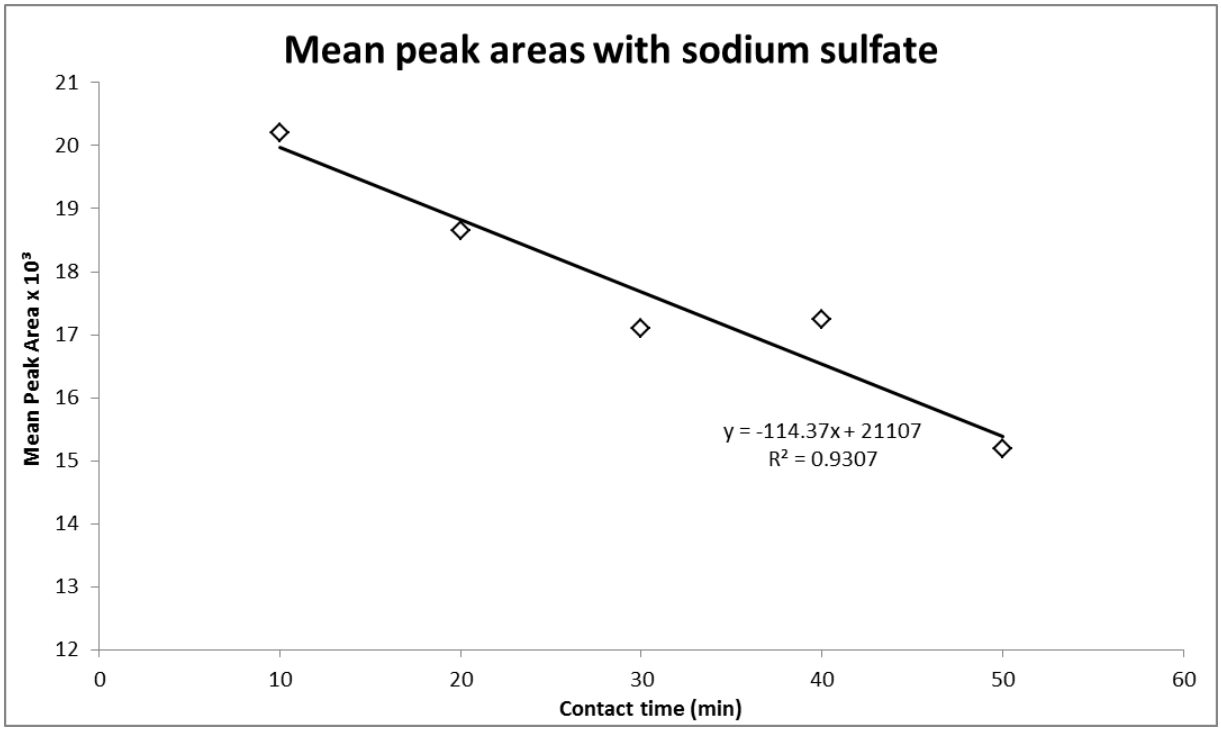

Figure 55: Mean peak areas obtained via chemical drying with sodium sulfate with varying contact times between eluate and the drying reagent showing a roughly linear trend in decreasing peak areas

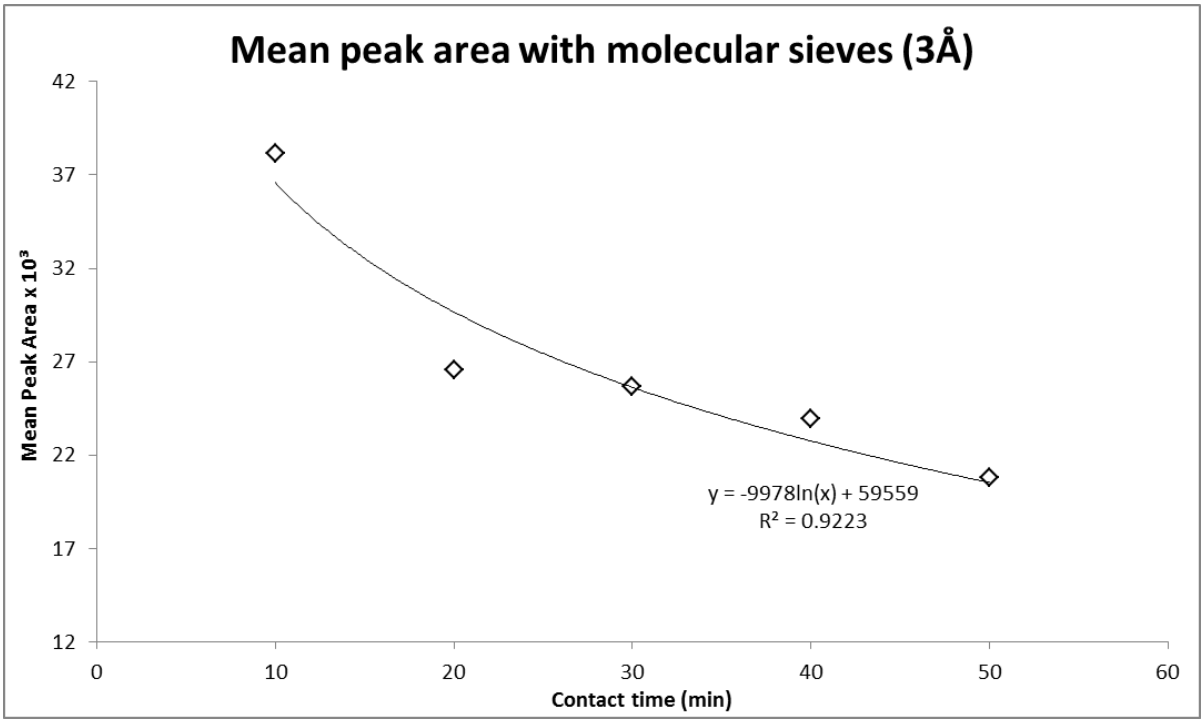

Figure 56: Mean peak areas obtained via chemical drying using molecular sieves with a diameter of 3Å and varying contact times between eluate and the drying reagent showing a roughly logarithmic trend in decreasing peak areas

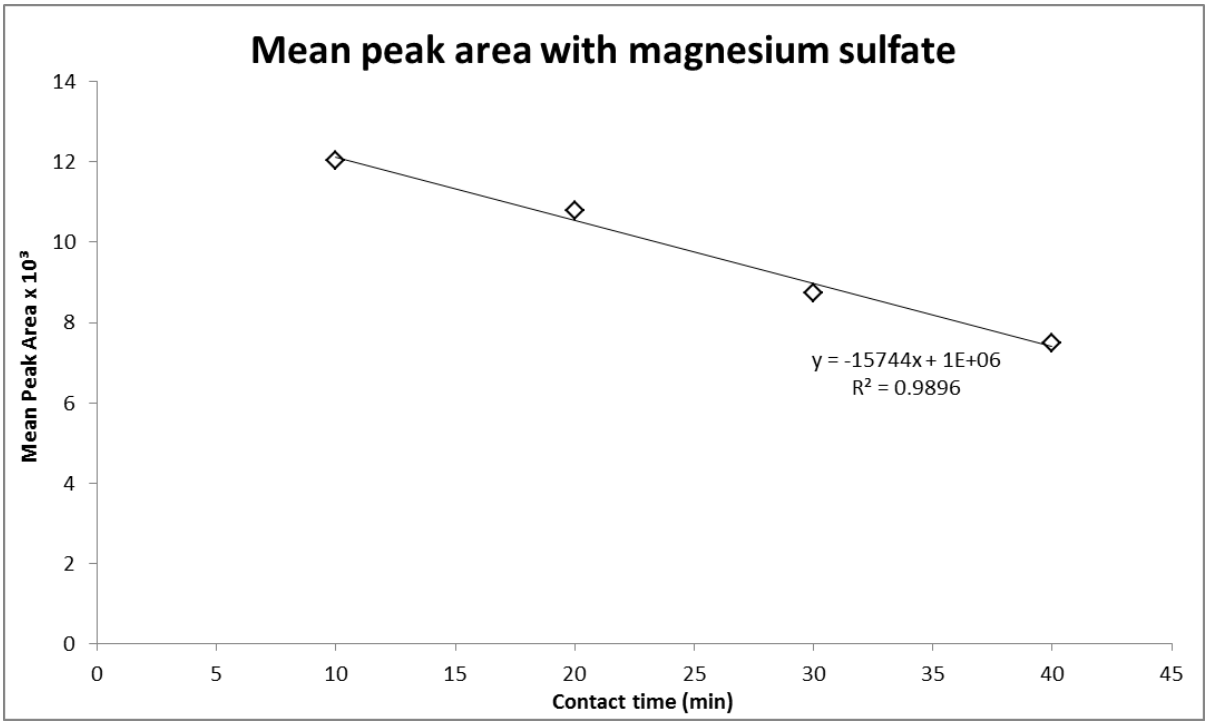

Figure 57: Mean peak areas obtained via chemical drying using magnesium sulfate and varying contact times between eluate and the drying reagent showing a roughly linear trend in decreasing peak areas

Considering the optimization of the selected evaporation procedure, temperature, nitrogen flow and time were the adjustable parameters. For the time being, the former two were kept constant at 50°C and 50 mL min^{-1} and the time was varied in order to test the effects of exhaustive (to dryness) and non-exhaustive (leaving a thin liquid film at the bottom of the vial) evaporation of the eluate.

The latter possibility turned out to be more effective, especially if sensitive analysis of the low molecular weight phenols was desired. Phenol for example, showed a significant decrease in detectable signal-to-noise ratios when the eluate was evaporated to dryness as depicted in Figure 58.

Practically, complete evaporation occurred after timeframes > 30 min, so that 25 min were selected as evaporation time, resulting in a remaining thin film of solvent at the bottom of the sample vial.

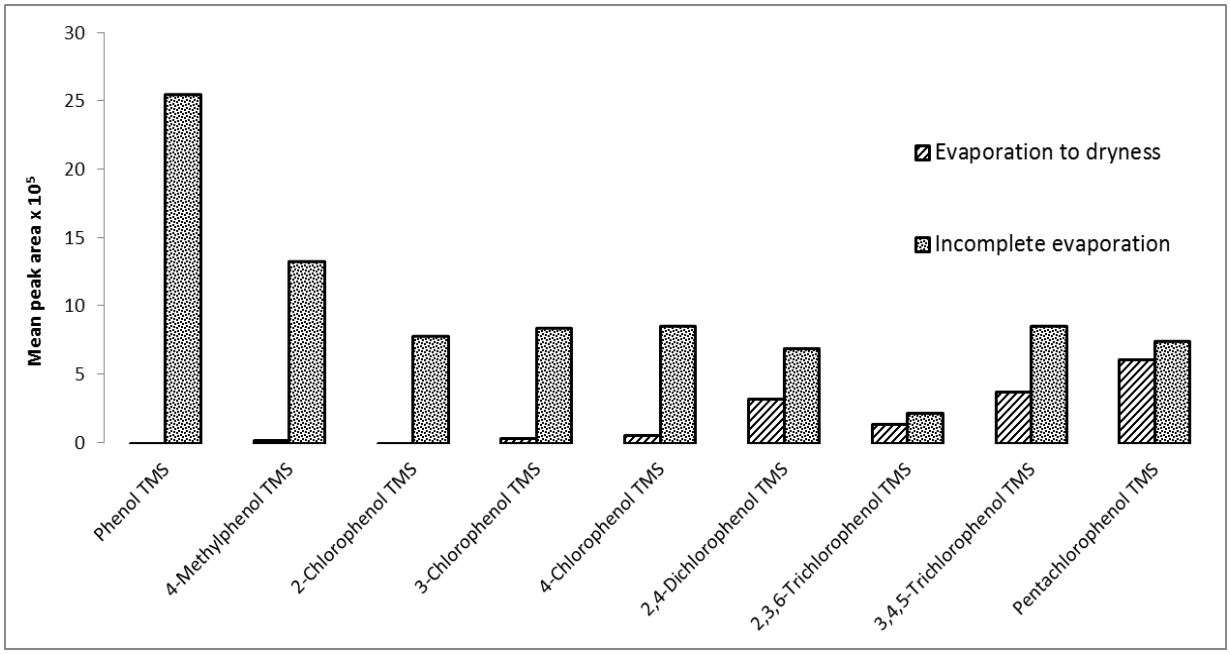

Figure 58: Mean peak areas obtained via exhaustive and non-exhaustive evaporation of the eluates prior to derivatization during evaluation of the ITSP method

In comparison to this evaporation procedure, the reagent-based drying options also resulted in poor repeatability. Figure 59 compares the mean RSDs for three evaluated options at the basis of a similar required amount of time (~ 30 min).

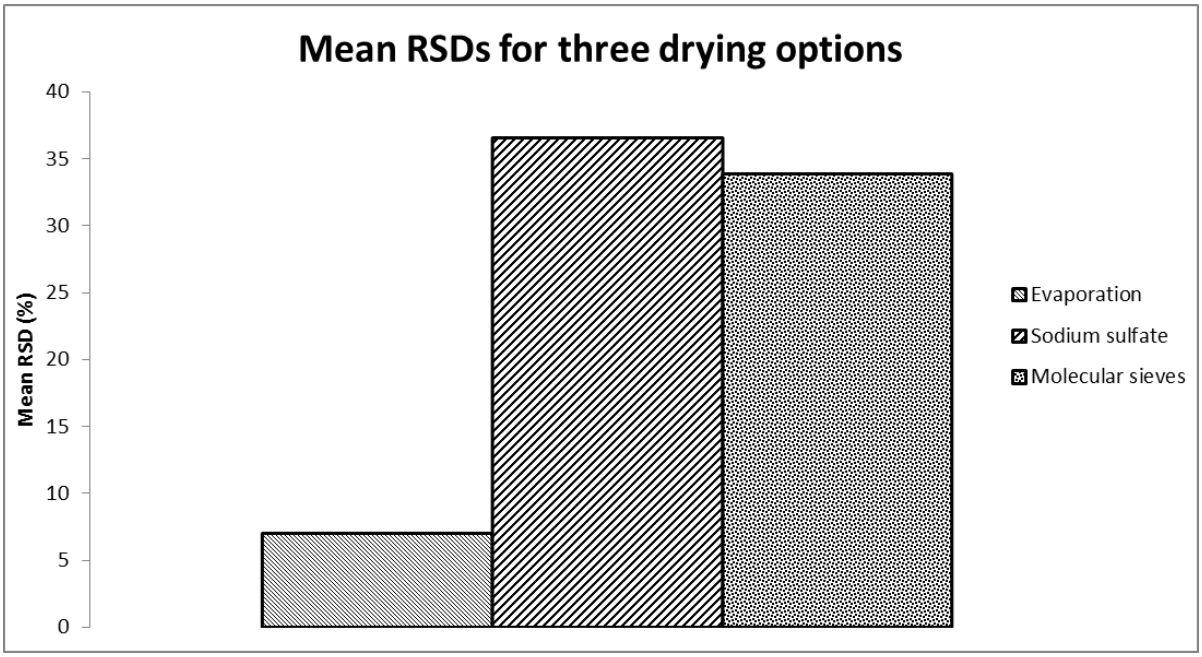

Figure 59: Mean RSDs for three evaluated drying options displayed according to a similar required timeframe of 30 min during evaluation of the ITSP method

Calibration. In order to compare the analytical potential of the ITSP option to other alternatives such as SPME[6], LODs and RSDs were determined with an initial calibration in the range of 100 to 0.01 µg L^{-1} using the internal standards mentioned under reagents and materials. Thereby a mean RSD of 7.01% and a mean LOD of approx. 0.7 µg L^{-1} were achieved with the detailed results being displayed in Table 30. Phenol d5 was used as the sole internal standard during evaluation of these data since 2-chlorophenol d4 gave erratic results when used as a basis for peak area normalizations.

Achieved RSDs and LODs are in good agreement to literature[111]. The manual derivatization and LLE procedure according to the German standard DIN 38407-27 for phenols and cresols, e.g., resulted in similar values without the involvement of automatization[108] (RSDs between 3.3 and 15.4%, and LODs in the range of 1.2 to 5 µg L^{-1}). Similarly, plausibility of results can be confirmed for the chlorophenols by comparing the results generated in this study to those given in DIN EN 12673[109] (detection range between 0.1 and 27.5 µg L^{-1} and RSDs between 5 and 22%).

Upon comparing these results, it should also be taken into account that those given in the official standards were generated using real-world samples in terms of groundwater or drinking water. Since the results presented in Table 30 were achieved using spiked lab water, it is effortlessly explainable why they are mostly better especially in terms of lower detection limits. On the other hand, further method optimization using ITSP might yield an even better performance.

Table 30: Results of an initial calibration for phenols extracted from water by automated MSPE via ITSP

Compound	LOD (µg L^{-1})	RSD (%)	Linear correlation
4-Methylphenol TMS	0.15	2.1	0.9998
2-Chlorophenol TMS	0.34	5.3	0.9999
3-Chlorophenol TMS	0.09	17.5	0.9999
4-Chlorophenol TMS	0.05	8.6	0.9992
2-Bromphenol TMS	0.58	8.0	0.9999
4-Chloro-3-methylphenol TMS	0.19	2.6	0.9998
2,4-Dichlorophenol TMS	0.20	2.5	0.9998
2,4,5-Trichlorophenol TMS	0.32	4.7	0.9999
2,3,5-Trichlorophenol TMS	0.04	5.4	0.9997
4-Chloro-2-methoxyphenol TMS	1.15	2.3	0.9970
Butylated hydroxytoluene	4.44	21.1	0.9998
2,3,6 Trichlorophenol TMS	0.67	10.6	0.9998
2,4,6 Trichlorophenol TMS	1.68	7.7	0.9998
3,4,5 Trichlorophenol TMS	0.13	2.4	0.9998
Pentachlorophenol TMS	0.43	4.5	0.9999

Whisky measurements. As exemplary real-world samples, a strongly phenolic and richly flavored Islay malt Scotch whisky (whisky 1) and a less intensively flavored malt whisky from the Scottish highlands (whisky 2) were examined using the previously presented parameters and the obtained calibration. The results shown in Table 31 correlate well with literature data[99] and indicate the presence of notable amounts of phenolic compounds in especially the former beverage. As stated in the introduction to this chapter, such compounds are important constituents of the characteristic whisky flavor.

Since whisky is, however, typically enjoyed in limited quantities, it is doubtful that the discovered concentrations are of any severe health concern, since these compounds are non-bioaccumulative and excreted without problems unless they surpass an acute toxicity threshold.

According to Conner et al., phenolic materials are only negligibly extracted from the cask material in case of scotch whisky[114]. It can therefore be assumed that the drying of the malt material over peat fires ("peating") and the storage conditions during maturation are the main influences on the concentrations of phenolic compounds in the final product. The casks of Islay malt whisky are stored in direct vicinity of the sea during maturation, potentially absorbing a multitude of additional compounds from the ambient air in comparison to the casks that are stored in the Scottish highlands.

The significance of the latter aspect was also confirmed by Nose et al. who pronounced the importance of warehouse conditions during whisky maturation[115]. The importance of the malt drying process may be indicated by the found differences between the two whiskys that were analyzed here, since whisky from the Scottish highland region is typically less "peated" compared to the Islay malts.

Phenolic compounds possess significant influence on taste[116]. Thus, a connection between the significantly smaller phenol concentrations found in whisky 2 and the fact that this brand is often described to be less intense in flavor can be assumed.

Table 31: Measurement results for the phenol content of Scotch whisky samples determined via the evaluated ITSP method

Compound name	Concentrations (µg L^{-1})	
	Whisky 1	Whisky 2
4-Methylphenol	1974	0
2-Chlorophenol	119	0.4
4-Chlorophenol	14.7	0
2-Bromophenol	0	6.4
4-Chloro-3-methylphenol	22.9	0
2,4-Dichlorophenol	13.1	0.1
2,3,5-Trichlorophenol	14.9	0.1
4-Chloro-2-methoxyphenol	314	8.1
2,3,4 Trichlorophenol	20.5	0.2
2,4,6 Trichlorophenol	0	4.8
3,4,5 Trichlorophenol	29.1	0.2
Pentachlorophenol	68.1	0
Summarized content	2591	20.3

5.4 Conclusions and outlook

The general concept of MSPE is appealing and ITSP seems to be an interesting option in this field. However, in its current form it is not without drawbacks. First of all residual water can hardly be avoided after sample loading and may disturb especially the subsequent derivatization. Carefully optimized eluate evaporations as described in this work may therefore often be mandatory. Another option would be a chemical drying step which has however proven unsuccessful for phenols in this work.

In addition, the required, additional instrumentation is more extensive than in case of, e.g., DLLME[104] and the costs of the consumables (ITSP cartridges, vials with 300 µL insert, derivatization reagent, syringes, solvents) are considerable as well. Cartridges are quite narrow and require utmost precision by the sampler to be hit in their center reliably. Without the specialized ITSP needle guides this is not possible and even the slightest deviations usually destroy the used syringes. Still, the transport of the cartridges via needle is often problematic and results in lost cartridges and an aborted measurement sequence.

Due to the constant risk of a cartridge overflow, usable flows are very small, resulting in long necessary timeframes for loading and elution. In combination with evaporation and derivatization steps this leads to overall sample preparation sequences that are considerably longer than GC analysis times leading to a loss of potential measuring time.

The problematic pre-conditioning of the cartridges is another issue in comparison to classical SPE solutions. Usually organic solvents are used as a first conditioning step in SPE methods in order to clean and activate the sorbent materials. Although it does not seem to have impeded the repeatability of the achieved results presented herein, the sensitivity of the method in terms of the extraction exhaustiveness may well be diminished if the conditioning and activation of the extraction phase is less than optimal.

The currently unavoidable dilution of the sample prior to injection (20 µL to 200 µL) is also a drawback in terms of achievable method sensitivity, especially when combined with the general downsizing (approx. factor 10) that the ITSP cartridges represent in comparison to classical SPE cartridges or disks[107]. The resulting loss of achievable signal intensities thereby equals approx. a factor of 100 compared to the undiluted sample.

So while it might currently still be preferable to use alternative techniques such as SPME whenever possible, ITSP may still have potential to be developed into a useful option for SPE-specific analytical demands automated by PAL-type samplers. In order to support this development, especially evaluations of the multitude of different available sorption phase materials would be beneficial. The focus of the presented project was method development and basic evaluation so that only exemplary cartridges provided by the manufacturer were used. The programmed script can serve as a universal basis for further implementations such as filtration of LLME-extracts which would also be realizable via ITSP. Further, smaller issues such as the less-than-optimal reliability of, e.g., the needle-transport processes with the PAL3 will possibly be worked out soon via upcoming firmware-updates for the system.

After further optimization of the general reliability of the ITSP option, another beneficial aspect may be the setup of a factorial design, in order to find optimal method parameters. The latter may enable further improvement of sensitivity and repeatability. The reason for this is that except for a few important parameters of the sample preparation method, other values were kept constant here.

5.5 Supporting information

PAL RTC program code. The following pages contain the program code that was used to control the automated sample preparation method presented in this chapter. Major code chapters were translated into third-level document captions in order to facilitate navigation to parts of interest. The program code is presented with only minor changes in order to improve the reproducibility of the results and does therefore not follow normal orthographic rules.

```
// Phenol Sample Preparation Script by Andreas Kremser (AK) Uni Due IAC MKII V2.6 151001

// Includes: ITSP SPE Cartridge Conditioning, Extraction with Dilutor Tool, Elution, Sample Transfer, Thermostatted Evaporation to Dryness, Derivatization, Vortex Mixing, Fillup, Repeated Vortex Mixing, Injection

// Further Features: Bottom Sensing on most Penetrations, Fast Injection, Safe Evaporation via Modified MHE-Tool, Temperature controlled Solvent Evaporation with optional Agitation

procedure General(
```

General program parameters

```
sampleRack:IRack,

intermediateRack:IRack,

elutionRack:IRack,

reagentVialRack:IRack,

sampleIndex:Integer[1..100]=1,

reagentVialIndex:Integer[1..100]=10,

syringeForDerivatization:IToolLiquid,

syringeForSampleInjection:IToolLiquid,

syringeForElution:IToolLiquid,

syringeForConditioning:IToolLiquid,

headspaceTool:IToolGas,
```

sampleVolume:Volume[1uL..10000uL]=1uL,

intermediateVolume:Volume[1uL..10000uL]=200uL,

postDryingTransferVolume:Volume[1uL..10000uL]=100uL,

sampleAspirateFlowRate:Flow[1uL/s..10000uL/s]=2uL/s,

reagentAspirateFlowRate:Flow[1uL/s..10000uL/s]=2uL/s,

solventAspirateFlowRate:Flow[1uL/s..10000uL/s]=5uL/s,

intermediateAspirateFlowRate:Flow[1uL/s..10000uL/s]=5uL/s,

sampleVialDispenseDepth:Length[5mm..50mm]=15mm,

dryingVialDispenseDepth:Length[5mm..50mm]=10mm,

waitAfterCompletion:Time[0min..500min]=0min,

Bottomsense & penetration parameters

bottomSenseSampleVial:Boolean{false=<Off>, true=<On>}=true,

bottomSenseReagentVial:Boolean{false=<Off>, true=<On>}=true,

bottomSenseIntermediateVial:Boolean{false=<Off>, true=<On>}=true,

bottomSenseDryingVial:Boolean{false=<Off>, true=<On>}=false,

heightFromBottomSampleVial:Length[0mm..32mm]=0.1mm,

heightFromBottomReagentVial:Length[0mm..32mm]=0.1mm,

heightFromBottomIntermediateVial:Length[0mm..60mm]=0.1mm,

heightFromBottomDryingVial:Length[0mm..60mm]=0.1mm,

sampleVialPenetrationDepth:Length[1mm..32mm]=25mm,

reagentVialPenetrationDepth:Length[1mm..32mm]=25mm,

intermediateVialPenetrationDepth:Length[1mm..70mm]=25mm,

dryingVialPenetrationDepth:Length[1mm..70mm]=15mm,

sampleVialPenetrationSpeed:Speed[1mm/s..75mm/s]=10mm/s,

reagentVialPenetrationSpeed:Speed[1mm/s..75mm/s]=10mm/s,

intermediateVialPenetrationSpeed:Speed[1mm/s..75mm/s]=10mm/s,

dryingVialPenetrationSpeed:Speed[1mm/s..75mm/s]=10mm/s,

fillupSolventSourcePenetrationSpeed:Speed[1mm/s..75mm/s]=10mm/s,

elutionSolventSourcePenetrationSpeed:Speed[1mm/s..75mm/s]=10mm/s,

conditioningSolventSourcePenetrationSpeed:Speed[1mm/s..75mm/s]=10mm/s,

Filling stroke parameters

sampleFillingStrokesCount:Integer[0..15]=4,

reagentFillingStrokesCount:Integer[0..15]=3,

intermediateFillingStrokesCount:Integer[0..15]=3,

fillupSolventFillingStrokesCount:Integer[0..15]=3,

postDryingFillingStrokesCount:Integer[0..15]=2,

conditioningSolventFillingStrokesCount:Integer[0..15]=3,

sampleFillingStrokesVolume:Volume[0µL..1000µL]=5µL,

reagentFillingStrokesVolume:Volume[0µL..1000µL]=10µL,

intermediateFillingStrokesVolume:Volume[0µL..1000µL]=100µL,

fillupSolventFillingStrokesVolume:Volume[0µL..1000µL]=200µL,

conditioningSolventFillingStrokesVolume:Volume[0µL..1000µL]=50µL,

postDryingFillingStrokesVolume:Volume[0µL..1000µL]=30µL,

sampleFillingStrokesAspirateFlowRate:Flow[2µL/s..100µL/s]=2µL/s,

reagentFillingStrokesAspirateFlowRate:Flow[2µL/s..100µL/s]=2µL/s,

fillupSolventFillingStrokesAspirateFlowRate:Flow[2µL/s..100µL/s]=10µL/s,

intermediateFillingStrokesAspirateFlowRate:Flow[2µL/s..100µL/s]=10µL/s,

postDryingFillingStrokesAspirateFlowRate:Flow[2µL/s..100µL/s]=3µL/s,

postDryingFillingStrokesDispenseFlowRate:Flow[2µL/s..100µL/s]=3µL/s,

conditioningSolventFillingStrokesAspirateFlowRate:Flow[2µL/s..100µL/s]=10µL/s,

sampleFillingStrokesPostAspirateDelay:Time[0s..10s]=1s,

reagentFillingStrokesPostAspirateDelay:Time[0s..10s]=1s,

intermediateFillingStrokesPostAspirateDelay:Time[0s..10s]=1s,

fillupSolventFillingStrokesPostAspirateDelay:Time[0s..10s]=3s,

postDryingFillingStrokesPostAspirateDelay:Time[0s..10s]=3s,

conditioningSolventFillingStrokesPostAspirateDelay:Time[0s..10s]=3s,

sampleFillingStrokesPostDispenseDelay:Time[0s..10s]=2s,

intermediateFillingStrokesPostDispenseDelay:Time[0s..10s]=2s,

reagentFillingStrokesPostDispenseDelay:Time[0s..10s]=2s,

postDryingFillingStrokesPostDispenseDelay:Time[0s..10s]=3s,

fillupSolventFillingStrokesPostDispenseDelay:Time[0s..10s]=2s,

conditioningSolventFillingStrokesPostDispenseDelay:Time[0s..10s]=2s,

delayAfterSampleFillingStrokes:Time[0s..10s]=1s,

delayAfterReagentFillingStrokes:Time[0s..10s]=1s,

delayAfterFillupSolventFillingStrokes:Time[0s..10s]=2s,

delayAfterConditioningSolventFillingStrokes:Time[0s..10s]=2s,

delayAfterPostDryingFillingStrokes:Time[0s..10s]=2s,

delayAfterIntermediateFillingStrokes:Time[0s..10s]=2s,

Syringe wash parameters

washStationDerivatizationSyringe:IWashStation,

washStationSampleSyringe:IWashStation,

washStationFillupSolventSyringe:IWashStation,

washCyclesDerivatizationSyringe:Integer[0..15]=1,

washCyclesSampleSyringe:Integer[0..15]=2,

washCyclesFillupSolventSyringe:Integer[0..15]=1,

washSolventStep1DerivatizationSyringe:Integer[1..4]=1,

washSolventStep1SampleSyringe:Integer[1..4]=1,

washSolventStep1FillupSolventSyringe:Integer[1..4]=1,

washSolventStep2DerivatizationSyringe:Integer[1..4]=2,

washSolventStep2SampleSyringe:Integer[1..4]=2,

washSolventStep2FillupSolventSyringe:Integer[1..4]=2,

wastePortDepthDerivatizationSyringeWashStation:Length[10mm..45mm]=15mm,

wastePortDepthSampleSyringeWashStation:Length[10mm..45mm]=15mm,

wastePortDepthFillupSolventSyringeWashStation:Length[10mm..45mm]=15mm,

percentageWashVolumeDerivatizationSyringe:Percentage[0%..110%]=110%,

percentageWashVolumeSampleSyringe:Percentage[0%..110%]=110%,

percentageWashVolumeFillupSolventSyringe:Percentage[0%..110%]=110%,

washAirGapVolumeDerivatizationSyringe:Percentage[0%..50%]=0%,

washAirGapVolumeSampleSyringe:Percentage[0%..50%]=0%,

washAirGapVolumeFillupSolventSyringe:Percentage[0%..50%]=0%,

Syringe rinse parameters

sampleRinseVolume:Volume[0µL..1000µL]=3µL,

reagentRinseVolume:Volume[0µL..1000µL]=3µL,

sampleRinseCycles:Integer[0..10]=1,

reagentRinseCycles:Integer[0..10]=1,

Sample injection parameters

injectionMode:Integer{0=<Normal>, 1=<Fast>}=1,

injector:IInjector,

gasChromatograph:GC,

injectorPenetrationDepth:Length[10mm..73mm]=45mm,

injectorPenetrationSpeed:Speed[2mm/s..200mm/s]=100mm/s,

injectionFlowRate:Flow[1µL/s..250µL/s]=100µL/s,

injectionSignalMode:Integer{0=<PreInject>, 1=<PlungerUp>, 2=<PlungerDown>, 3=<None>}=2,

preInjectionDwellTime:Time[0s..15s]=0s,

postInjectionDwellTime:Time[0s..15s]=0s,

Derivatization parameters

vortexMixer:VortexMixer{none, all VortexMixer},

vortexFrequency:Frequency[100rpm..3000rpm]=1200rpm,

vortexTime:Time[0s..600s]=60s,

vortexTime2:Time[0s..600s]=10s,

derivatizationTime:Time[0min..120min]=0min,

postDispenseDwellTime:Time[0s..600s]=2s,

reagentVolume:Volume[1uL..10000uL]=10uL,

Elution & drying parameters

blowThroughFlowRate:Flow[1uL/s..1000uL/s]=200uL/s,

airAspirateFlowRate:Flow[1uL/s..1000uL/s]=100uL/s,

elutionSolventSource:IMoveTarget{all ITray, all LargeVolumeWashStation, all StandardWashStation, all LargeVolumeSolventStation},

elutionSolventIndex:Integer[1..96],

elutionSolventFlowRate:Flow[1uL/s..10000uL/s]=20uL/s,

elutionSolventVolume:Volume[1uL..10000uL]=500uL,

elutionSolventAspirateFlowRate:Flow[0.5µL/s..100µL/s]=20µL/s,

elutionSolventSourcePenetrationDepth:Length[5mm..44mm]=40mm,

elutionSolventPostDispenseDelay:Time[0s..60s]=5s,

vortexTimeDrying:Time[0s..600s]=60s,

dryingTime:Time[0min..120min]=60min,

evaporationTime:Time[0min..120min]=20min,

mheAdapter:MheAdapter,

incubationTime:Time[0.1min..600min]=0.5min,

incubationTemperature:Temperature[30°C..200°C]=35°C,

waitForReadinessAgitator:Boolean=true,

agitatorSpeed:Frequency[60rpm..750rpm]=400rpm,

agitatorOnTime:Time[1s..600s]=5s,

agitatorOffTime:Time[0s..600s]=2s,

agitator:Agitator,

agitatorTempTolerance:DeltaTemperature = 3dK,

agitationDuringIncubation:Boolean{false=<Off>, true=<On>}=false,

evaporationPenetrationDepth:Length[1mm..32mm]=15mm,

evaporationPenetrationSpeed:Speed[1mm/s..75mm/s]=10mm/s,

fillupSolventVolume:Volume[0µL..1000µL]=100µL,

fillupSolventSource:IMoveTarget{all ITray, all LargeVolumeWashStation, all StandardWashStation, all LargeVolumeSolventStation},

fillupSolventIndex:Integer[1..96],

fillupSolventFlowRate:Flow[1uL/s..10000uL/s]=20uL/s,

fillupSolventAspirateFlowRate:Flow[0.5µL/s..100µL/s]=20µL/s,

fillupSolventSourcePenetrationDepth:Length[5mm..44mm]=40mm,

ITSP parameters

cartridgeTrayITSP:IRack,

toolDilutor:ToolDilutor_53,

rackITSPCartridge:IRack,

cartridgeIndex:Integer[1..54],

sampleLoadVolume:Volume[0.001mL..1000mL]=5mL,

sampleLoadFlowRate:Flow[0.5µL/s..100µL/s]=5µL/s,

dilutorCartridgePenetrationDepth:Length[5mm..44mm]=20mm,

dilutorSamplePort:Integer[1..6],

dilutorRinsePort:Integer[1..6],

dilutorWasteTarget:IWashStation,

dilutorWasteTargetPenetrationDepth:Length[5mm..44mm]=15mm,

dilutorRinseFlowRate:Flow[0.5µL/s..100µL/s]=20µL/s,

dilutorPreRinseVolume:Volume[1mL..100mL]=3mL,

dilutorPostRinseVolume:Volume[1mL..100mL]=5mL,

dilutorPenetrationSpeed:Speed[2mm/s..200mm/s]=20mm/s,

conditioningSolventSource:IMoveTarget{all ITray, all LargeVolumeWashStation, all StandardWashStation, all LargeVolumeSolventStation},

conditioningSolventIndex:Integer[1..96],

conditioningSolventVolume:Volume[1µL..1000µL]=200µL,

conditioningSolventAspirateFlowRate:Flow[0.5µL/s..100µL/s]=20µL/s,

conditioningSolventDispenseFlowRate:Flow[0.5µL/s..100µL/s]=2µL/s,

conditioningSolventSourcePenetrationDepth:Length[5mm..44mm]=40mm

)

Volatile programming values

```
var

vial:IVolatileVial

vialDrying:IVolatileVial

cartridge:IVolatileObject

n:Integer

agitatorIndex:Integer

dilutor:Dilutor

toolPort:Integer

wastePort:Integer


// Procedures
```

Rinsing procedures

```
procedure RinseSampleSyringe()

begin

for n=1 to sampleRinseCycles do

MoveToObject( target=sampleRack, index=sampleIndex)

if bottomSenseSampleVial then

PenetrateWithBottomSense(target=sampleRack,index=sampleIndex,
heightFromBottom=heightFromBottomSampleVial, speed=sampleVialPenetrationSpeed)

else

PenetrateObject(target=sampleRack,index=sampleIndex,depth=sampleVialPenetrationDepth,
speed=sampleVialPenetrationSpeed)

end

AspirateSyringe( volume=sampleRinseVolume, flowRate=sampleAspirateFlowRate)

LeaveObject(leaveDrawerOpen=true)
```

```
MoveToObject( target=washStationSampleSyringe.Waste)

PenetrateObject( target=washStationSampleSyringe.Waste, speed=sampleVialPenetrationSpeed)

EmptySyringe( )

LeaveObject(leaveDrawerOpen=true)

end

end

procedure RinseDerivatizationSyringe()

begin

for n=1 to reagentRinseCycles do

MoveToObject( target=reagentVialRack, index=reagentVialIndex)

if bottomSenseReagentVial then PenetrateWithBottomSense( target=reagentVialRack, index=reagentVialIndex,
heightFromBottom=heightFromBottomReagentVial, speed=reagentVialPenetrationSpeed)

else

PenetrateObject(target=reagentVialRack,index=reagentVialIndex,depth=reagentVialPenetrationDepth,
speed=reagentVialPenetrationSpeed)

end

AspirateSyringe( volume=reagentRinseVolume, flowRate=reagentAspirateFlowRate)

LeaveObject(leaveDrawerOpen=true)

MoveToObject( target=washStationDerivatizationSyringe.Waste)

PenetrateObject( target=washStationDerivatizationSyringe.Waste, speed=reagentVialPenetrationSpeed)

EmptySyringe( )

LeaveObject(leaveDrawerOpen=true)

end

end
```

Wash step procedures

```
procedure WashSampleSyringe(solventIndex:Integer)

begin

CleanSyringe(washSource=washStationSampleSyringe,washIndex=solventIndex,
wasteTarget=washStationSampleSyringe, wastePenetrationDepth=wastePortDepthSampleSyringeWashStation,

washVolume=percentageWashVolumeSampleSyringe,
washAirGapVolume=washAirGapVolumeSampleSyringe, cycles=washCyclesSampleSyringe)

end

procedure WashDerivatizationSyringe(solventIndex:Integer)

begin

CleanSyringe(washSource=washStationDerivatizationSyringe,washIndex=solventIndex,
wasteTarget=washStationDerivatizationSyringe,
wastePenetrationDepth=wastePortDepthDerivatizationSyringeWashStation,

washVolume=percentageWashVolumeDerivatizationSyringe,
washAirGapVolume=washAirGapVolumeDerivatizationSyringe, cycles=washCyclesDerivatizationSyringe)

end

procedure WashElutionSyringe(solventIndex:Integer)

begin

CleanSyringe(washSource=washStationFillupSolventSyringe,washIndex=solventIndex,
wasteTarget=washStationFillupSolventSyringe,
wastePenetrationDepth=wastePortDepthFillupSolventSyringeWashStation,

washVolume=percentageWashVolumeFillupSolventSyringe,
washAirGapVolume=washAirGapVolumeFillupSolventSyringe, cycles=washCyclesFillupSolventSyringe)

end
```

Main code

begin

vial=sampleRack[sampleIndex]

cartridge=rackITSPCartridge[sampleIndex]

dilutor=toolDilutor.Dilutor

toolPort=dilutor.ValveType.ToolPositionPort

wastePort=dilutor.ValveType.PlungerHomingPositionPort

ITSP with dilutor tool

SetStatus(key="GetTool")

ChangeTool(tool=syringeForConditioning)

MoveToObject(target=conditioningSolventSource, index=conditioningSolventIndex)

PenetrateObject(target=conditioningSolventSource,index=conditioningSolventIndex, depth=conditioningSolventSourcePenetrationDepth, speed=conditioningSolventSourcePenetrationSpeed)

FillingStrokes(volume=conditioningSolventFillingStrokesVolume, aspirateFlowRate=conditioningSolventFillingStrokesAspirateFlowRate,

pullupDelay=conditioningSolventFillingStrokesPostDispenseDelay,dispenseDelay=conditioningSolventFillingStrokesPostAspirateDelay, count=conditioningSolventFillingStrokesCount)

Wait(time=delayAfterConditioningSolventFillingStrokes)

AspirateSyringe(volume=conditioningSolventVolume, flowRate=conditioningSolventAspirateFlowRate)

LeaveObject()

MoveToObject(target=rackITSPCartridge, index=sampleIndex)

PenetrateObject(target=rackITSPCartridge,index=sampleIndex,depth=dilutorCartridgePenetrationDepth, speed=conditioningSolventSourcePenetrationSpeed)

EmptySyringe(flowRate=conditioningSolventDispenseFlowRate)

LeaveObject()

SetStatus(key="GetTool")

ChangeTool(tool=toolDilutor)

MoveToObject(target=dilutorWasteTarget.Waste)

PenetrateObject(target=dilutorWasteTarget.Waste,speed=dilutorPenetrationSpeed, depth=dilutorWasteTargetPenetrationDepth)

DeliverLiquidDilutor(dilutor=dilutor,volume=dilutorPreRinseVolume, dispenseFlowRate=dilutorRinseFlowRate, solventPort=dilutorSamplePort, deliveryPort=toolPort)

Wait(time=2s)

Depenetrate()

LeaveObject()

MoveToObject(target=rackITSPCartridge, index=sampleIndex)

PenetrateObject(target=rackITSPCartridge,index=sampleIndex,depth=dilutorCartridgePenetrationDepth, speed=dilutorPenetrationSpeed)

DeliverLiquidDilutor(dilutor=dilutor, volume=sampleLoadVolume, dispenseFlowRate=sampleLoadFlowRate, solventPort=dilutorSamplePort, deliveryPort=toolPort)

Wait(time=2s)

Depenetrate()

MoveRelative(movementZ=-2mm, forceDirectMovement=true)

MoveRelative(movementY=-5mm, forceDirectMovement=true)

LeaveObject()

MoveToObject(target=dilutorWasteTarget.Waste)

PenetrateObject(target=dilutorWasteTarget.Waste,speed=dilutorPenetrationSpeed, depth=dilutorWasteTargetPenetrationDepth)

DeliverLiquidDilutor(dilutor=dilutor,volume=dilutorPostRinseVolume, dispenseFlowRate=dilutorRinseFlowRate, solventPort=dilutorRinsePort, deliveryPort=toolPort)

Wait(time=2s)

Depenetrate()

LeaveObject()

Cartridge elution

SetTemperature(target=agitator,temperature=incubationTemperature,wait=waitForReadinessAgitator, tolerance=agitatorTempTolerance)

ChangeTool(tool=syringeForElution)

AspirateSyringe(volume=1mL, flowRate=airAspirateFlowRate)

MoveToObject(target=rackITSPCartridge, index=sampleIndex)

PenetrateObject(target=rackITSPCartridge,index=sampleIndex,depth=dilutorCartridgePenetrationDepth, speed=dilutorPenetrationSpeed)

EmptySyringe(flowRate=blowThroughFlowRate)

Depenetrate()

AspirateSyringe(volume=1mL, flowRate=airAspirateFlowRate)

MoveToObject(target=rackITSPCartridge, index=sampleIndex)

PenetrateObject(target=rackITSPCartridge,index=sampleIndex,depth=dilutorCartridgePenetrationDepth, speed=dilutorPenetrationSpeed)

EmptySyringe(flowRate=blowThroughFlowRate)

Depenetrate()

AspirateSyringe(volume=1mL, flowRate=airAspirateFlowRate)

MoveToObject(target=rackITSPCartridge, index=sampleIndex)

PenetrateObject(target=rackITSPCartridge,index=sampleIndex,depth=dilutorCartridgePenetrationDepth, speed=dilutorPenetrationSpeed)

EmptySyringe(flowRate=blowThroughFlowRate)

Depenetrate()

MoveRelative(movementZ=-2mm, forceDirectMovement=true)

MoveRelative(movementY=-5mm, forceDirectMovement=true)

LeaveObject()

MoveToObject(target=fillupSolventSource, index=fillupSolventIndex)

PenetrateObject(target=fillupSolventSource,index=fillupSolventIndex, depth=fillupSolventSourcePenetrationDepth, speed=fillupSolventSourcePenetrationSpeed)

FillingStrokes(volume=fillupSolventFillingStrokesVolume,
aspirateFlowRate=fillupSolventFillingStrokesAspirateFlowRate,pullupDelay=fillupSolventFillingStrokesPostDispenseDelay,dispenseDelay=fillupSolventFillingStrokesPostAspirateDelay,
count=fillupSolventFillingStrokesCount)

Wait(time=delayAfterFillupSolventFillingStrokes)

AspirateSyringe(volume=elutionSolventVolume, flowRate=fillupSolventAspirateFlowRate)

LeaveObject()

MoveToObject(target=rackITSPCartridge, index=sampleIndex)

PenetrateObject(target=rackITSPCartridge,index=sampleIndex,depth=dilutorCartridgePenetrationDepth,
speed=dilutorPenetrationSpeed)

TransportVial(source=cartridge, destination=elutionRack, destinationIndex=sampleIndex, leaveObject=false, home="Source")

EmptySyringe(flowRate=elutionSolventFlowRate)

Wait(time=elutionSolventPostDispenseDelay)

TransportVialHome(vial=cartridge, leaveObject=false)

Depenetrate()

MoveRelative(movementZ=-2mm, forceDirectMovement=true)

MoveRelative(movementY=-5mm, forceDirectMovement=true)

LeaveObject()

AspirateSyringe(volume=1000µL, flowRate=airAspirateFlowRate)

MoveToObject(target=rackITSPCartridge, index=sampleIndex)

PenetrateObject(target=rackITSPCartridge,index=sampleIndex,depth=dilutorCartridgePenetrationDepth,
speed=dilutorPenetrationSpeed)

TransportVial(source=cartridge, destination=elutionRack, destinationIndex=sampleIndex, leaveObject=false, home="Source")

EmptySyringe(flowRate=blowThroughFlowRate)

Wait(time=elutionSolventPostDispenseDelay)

TransportVialHome(vial=cartridge, leaveObject=false)

Depenetrate()

MoveRelative(movementZ=-2mm, forceDirectMovement=true)

MoveRelative(movementY=-5mm, forceDirectMovement=true)

LeaveObject()

Sample transfer

MoveToObject(target=intermediateRack, index=sampleIndex)

if bottomSenseIntermediateVial then

PenetrateWithBottomSense(target=intermediateRack,index=sampleIndex,
heightFromBottom=heightFromBottomIntermediateVial, speed=intermediateVialPenetrationSpeed)

else

PenetrateObject(target=intermediateRack,index=sampleIndex,depth=intermediateVialPenetrationDepth,
speed=intermediateVialPenetrationSpeed)

end

FillingStrokes(volume=intermediateFillingStrokesVolume,
aspirateFlowRate=intermediateFillingStrokesAspirateFlowRate,pullupDelay=intermediateFillingStrokesPostDispenseDelay,dispenseDelay=intermediateFillingStrokesPostAspirateDelay,
count=intermediateFillingStrokesCount)

Wait(time=delayAfterIntermediateFillingStrokes)

AspirateSyringe(volume=intermediateVolume, flowRate=intermediateAspirateFlowRate)

LeaveObject( )

MoveToObject(target=sampleRack, index=sampleIndex)

PenetrateObject(target=sampleRack,index=sampleIndex,depth=sampleVialDispenseDepth,
speed=sampleVialPenetrationSpeed)

EmptySyringe()

LeaveObject()

WashElutionSyringe(solventIndex=washSolventStep2FillupSolventSyringe)

WashElutionSyringe(solventIndex=washSolventStep1FillupSolventSyringe)

Evaporation procedure

```
agitatorIndex = Allocate(resource=agitator)

ChangeTool( tool=syringeForSampleInjection)

TransportVial( source=vial, destination=agitator, destinationIndex=agitatorIndex, home="Source")

if agitationDuringIncubation<>false then

SetAgitator(agitator=agitator,state="On",speed=agitatorSpeed,onTime=agitatorOnTime,
offTime=agitatorOffTime)

end

Wait ( time=incubationTime)

ChangeTool( tool=headspaceTool)

PickToolAdapter( toolAdapter=mheAdapter)

StartPurgeSyringe()

Wait( time=10s)

if agitationDuringIncubation<>false then

SetAgitator( agitator=agitator, state="Off")

end

MoveToObject( target=vial)

PenetrateObject( target=vial, depth=evaporationPenetrationDepth, speed=evaporationPenetrationSpeed)

Wait( time=evaporationTime)

LeaveObject()

StopPurgeSyringe()

ParkToolAdapter()

WaitOverlap( time=31s)

SetBusy( time=31s, resource=injector)

Release( position=agitatorIndex, resource=agitator)
```

Derivatization

ChangeTool(tool=syringeForDerivatization)

TransportVialHome(vial=vial)

WashDerivatizationSyringe(solventIndex=washSolventStep1DerivatizationSyringe)

WashDerivatizationSyringe(solventIndex=washSolventStep2DerivatizationSyringe)

RinseDerivatizationSyringe()

MoveToObject(target=reagentVialRack, index=reagentVialIndex)

if bottomSenseReagentVial then

PenetrateWithBottomSense(target=reagentVialRack,index=reagentVialIndex, heightFromBottom=heightFromBottomReagentVial, speed=reagentVialPenetrationSpeed)

else

PenetrateObject(target=reagentVialRack,index=reagentVialIndex,depth=reagentVialPenetrationDepth, speed=reagentVialPenetrationSpeed)

end

FillingStrokes(volume=reagentFillingStrokesVolume, aspirateFlowRate=reagentFillingStrokesAspirateFlowRate,pullupDelay=reagentFillingStrokesPostDispenseDelay,dispenseDelay=reagentFillingStrokesPostAspirateDelay, count=reagentFillingStrokesCount)

Wait(time=delayAfterReagentFillingStrokes)

AspirateSyringe(volume=reagentVolume, flowRate=reagentAspirateFlowRate)

LeaveObject( )

MoveToObject(target=sampleRack, index=sampleIndex)

PenetrateObject(target=sampleRack,index=sampleIndex,depth=sampleVialDispenseDepth, speed=sampleVialPenetrationSpeed)

EmptySyringe()

Wait(time=postDispenseDwellTime)

LeaveObject()

WashDerivatizationSyringe(solventIndex=washSolventStep1DerivatizationSyringe)

WashDerivatizationSyringe(solventIndex=washSolventStep2DerivatizationSyringe)

```
if vortexMixer<>none then

VortexVial( source=vial, vortexMixerSpeed=vortexFrequency,vortexMixer=vortexMixer, time=vortexTime)

TransportVialHome( vial=vial)

MoveToHome( )

end

SetStatus(key="DebugMessage_1Value", "Derivatization time", derivatizationTime)

Wait(time=derivatizationTime)

// Fillup

ChangeTool( tool=syringeForElution)

MoveToObject( target=fillupSolventSource, index=fillupSolventIndex)

PenetrateObject(target=fillupSolventSource,index=fillupSolventIndex,
depth=fillupSolventSourcePenetrationDepth, speed=fillupSolventSourcePenetrationSpeed)

FillingStrokes(volume=fillupSolventFillingStrokesVolume,
aspirateFlowRate=fillupSolventFillingStrokesAspirateFlowRate,pullupDelay=fillupSolventFillingStrokesPostDi
spenseDelay,dispenseDelay=fillupSolventFillingStrokesPostAspirateDelay,
count=fillupSolventFillingStrokesCount)

Wait( time=delayAfterFillupSolventFillingStrokes)

AspirateSyringe( volume=fillupSolventVolume, flowRate=fillupSolventAspirateFlowRate)

LeaveObject()

MoveToObject( target=sampleRack, index=sampleIndex)

PenetrateObject(target=sampleRack,index=sampleIndex,depth=sampleVialDispenseDepth,
speed=sampleVialPenetrationSpeed)

EmptySyringe()

LeaveObject()

WashElutionSyringe(solventIndex=washSolventStep1FillupSolventSyringe)
```

Sample Injection

ChangeTool(tool=syringeForSampleInjection)

if vortexMixer<>none then

VortexVial(source=vial, vortexMixerSpeed=vortexFrequency,vortexMixer=vortexMixer, time=vortexTime2)

TransportVialHome(vial=vial)

MoveToHome( )

end

WashSampleSyringe(solventIndex=washSolventStep1SampleSyringe)

WashSampleSyringe(solventIndex=washSolventStep2SampleSyringe)

RinseSampleSyringe()

MoveToObject(target=sampleRack, index=sampleIndex)

if bottomSenseSampleVial then

PenetrateWithBottomSense(target=sampleRack,index=sampleIndex,
heightFromBottom=heightFromBottomSampleVial, speed=sampleVialPenetrationSpeed)

else

PenetrateObject(target=sampleRack,index=sampleIndex,depth=sampleVialPenetrationDepth,
speed=sampleVialPenetrationSpeed)

end

FillingStrokes(volume=sampleFillingStrokesVolume,
aspirateFlowRate=sampleFillingStrokesAspirateFlowRate,pullupDelay=sampleFillingStrokesPostDispenseDela
y,dispenseDelay=sampleFillingStrokesPostAspirateDelay, count=sampleFillingStrokesCount)

Wait(time=delayAfterSampleFillingStrokes)

AspirateSyringe(volume=sampleVolume, flowRate=sampleAspirateFlowRate)

LeaveObject( )

if injectionMode == 0 then

InjectSampleGC(injector=injector, penetrationDepth=injectorPenetrationDepth,

penetrationSpeed=injectorPenetrationSpeed,preDelay=preInjectionDwellTime,

```
flowRate=injectionFlowRate, postDelay=postInjectionDwellTime,
injectedSignal=gasChromatograph.Injected, injectedSignalMode=injectionSignalMode)
end
if injectionMode == 1 then
FastInjectSampleGC( injector=injector,penetrationDepth=injectorPenetrationDepth,
injectedSignal=gasChromatograph.Injected)
end
WashSampleSyringe(solventIndex=washSolventStep1SampleSyringe)
WashSampleSyringe(solventIndex=washSolventStep2SampleSyringe)
MoveToHome ( )
Wait( time=waitAfterCompletion)
end
```

6 General conclusions and outlook

Modern analytical demands continue to develop into a direction, where reliable automation, sensitivity, repeatability and flexibility are the key parameters to successful sample preparation[1]. It is therefore mandatory to continue the development of both new and proven microextraction techniques alike, in order to fulfill these criteria in increasingly complex analytical systems[2]. Individual features of the available solutions have to be evaluated and weighted against each other in order to identify their most appropriate field of use. Besides extensive comparisons of established methods, especially two new options in the field of microextraction techniques for automated sample preparation were evaluated during the course of this work: PAL SPME Arrow[7] and ITSP MSPE cartridges.

The former was accompanied throughout its development process and also compared to available, comparative techniques in order to assess its analytical potential. Obtained results for both techniques were promising. PAL SPME Arrow augments the proven concept of SPME with improved mechanical reliability and sensitivity - the two aspects in which the technique still had room for improvement[7]. And while SPME-related sampling methods with enlarged sorption phase volumes were already developed with the concept of SBSE[19,20], the possibility of a straightforward and full automation of a large-volume SPME device, using mostly off-the-shelf instrumentation, has been shown for the first time in this work. The used agitation station was, however, still a self-constructed solution here, which delivered effective mixing by magnetic stirring of the samples. A new mixing solution (CTC Analytics HeatEx Stirrer), which is optimized for PAL SPME Arrow will be released soon and claims to combine an even more effective mixing with a facilitated sample preparation, since no stir-bars have to be added to the samples anymore. Further studies in the context of PAL SPME Arrow could therefore involve this new device and start with an evaluation and comparison of optimal extraction times for different, available agitation options. In addition, the intensities/velocities of agitation which are most suitable for either immersion- or headspace extraction could be determined.

Headspace analysis is very attractive and widely used due to inherent matrix separation[9,12,14]. Despite this, a comprehensive comparison of headspace-based sample preparation techniques by measured data on one and the same instrument was lacking so far. This work remediates this lack to a large degree and may facilitate a systematic and unbiased classification of the investigated techniques according to their individual properties.

Concerning the general classification of headspace techniques that was proposed in the introduction to this work, it might be interesting to include stripping techniques such as purge & trap into subsequent studies. Especially in terms of achievable extraction yields, such an extension of the comparison that was carried out here may either confirm or contest the assumption that one group of dynamic enrichment techniques is sufficient due to limited differences in method effectiveness. Should stripping techniques indeed provide additional benefit in terms of, e.g., a more rapid and more exhaustive extraction of analytes, a next step could be to attempt the implementation of a stripping technique on a PAL-type sampler. In case of a success, this could mitigate the cost and laboratory footprint that is connected to, e.g., purge & trap instrumentation[8]. Furthermore, the potential for further optimization of each individual headspace analysis method may be determined via a factorial design study. As shown before for ITEX, such studies can yield improved results and it might be interesting if this is the case to a different degree for the methods investigated herein[61].

Not only the mentioned new microextraction options, but also miniaturized, automated versions of classical sample preparation techniques such as LLE have the potential to replace the original, manual methods[117,118]. The use of automation improves the user-independence of measurement results, i.e., repeatability and reproducibility. Further optimized aspects due to the combination of miniaturization with automation are safety and efficient use of measuring time, staff, organic solvents and money.

The flexibility of novel autosampler generations complement the possibilities granted by automated, classical sample preparation and microextraction techniques. Examples here are automated tool changes and new modules such as automated evaporation of samples and vortex mixing. These new capabilities permit the automation of an increased number of previously manual sample preparation procedures. Also possible are all sorts of imaginable combinations of microextraction techniques with additional, automated sample preparation steps such as derivatization by, e.g., acetylation with subsequent HS-SPME extraction, which has previously proven successful for phenols in aqueous samples[6].

This synergy between miniaturization and automation was demonstrated in chapter 5 of this work, where an automated SPE method for flexible volumes of water samples was complemented by on-line evaporation and derivatization of samples. To our knowledge, this was the first time that such a comprehensive SPE approach was fully automated on a PAL-type sampler.

The experiences gained in this part of the presented work can help to optimize the reliability of the involved devices and also to develop similar methods in the future. The work that has been described in that chapter is also usable for a multitude of different sample preparations such as the mentioned DLLME with subsequent filtration of the organic fraction, e.g., in case of biological or wastewater samples. Alongside the possibilities of the novel ITSP cartridges, this flexibility may enable automation of many of the numerous, well-established SPE methods by PAL-type samplers. Besides notable potential, also remaining difficulties were found that require improvement of the method. An example for such optimization potential would be the problematic sealing of the dilutor tool and the ITSP cartridges during sample loading as described in chapter 5. Future studies in this context may benefit from the findings in this work, resulting in, e.g., faster sample extraction due to larger flows, which will be possible when an improved cartridge sealing has been implemented. Another example here would be the extensively optimized evaporation procedure that was described herein.

The PAL3 program script that was created for the automation of the procedures described in chapter 5 is very flexible and modular, so that it can be adapted to perform a manifold of different, automated sample preparation procedures on this type of autosamplers. This flexibility provides the basis for subsequent studies. These may for example again involve the setup of a factorial design in order to further optimize method parameters[61].

When the problems described in the conclusions of chapter 5 are overcome, possibilities for automated sample preparation on similar systems can be manifold. One example would be fully automated calibration sequences, prepared by the instrument itself from the desired solvents and analytes. Using intelligent, experiment-data-feedback and instrument control software such as Chronos EBIS (Experiment-basierte Instrumentensteuerung or experiment-based instrument control) (Axel Semrau GmbH, Sprockhövel, Germany), instruments now develop the capability of a true self-control according to generated results. This means that the instrument is able to react to measured data by adjusting its method parameters for the subsequent measurements. Should a real-world sample, e.g., exhibit analyte concentrations outside the calibration range, the instrument could either repeat the sample measurement after automatically diluting it (in case of a too high sample concentration) or create a new calibration that fits the necessary concentration range (in case of a too low sample concentration).

Initially suggested measurement parameters for individual samples could thereby be registered to two-dimensional barcodes that are applicable to the sample vials and readable by the autosampler. The latter functionality is already employed, however, primarily for surveillance of a correct relation between prepared and measured samples in order to avoid mix-ups.

This growing intelligence of the instrument control software may in future synergize with the progress in automation capabilities and sample preparation techniques, which was the subject of this work. It is the profound opinion of the author that the widespread adoption of such new options in routine laboratories still holds exceptional potential for the future of analytical chemistry all over the world.

7 Appendix

7.1 Abbreviations and symbols

BHT	-	Butylated hydroxytoluene
BPA	-	Bisphenol A
BTEX	-	Benzene, toluene, ethylbenzene and xylenes
DHS	-	Dynamic headspace
DI	-	Direct immersion
DLLME	-	Dispersive liquid-liquid microextraction
EBIS	-	Experiment-based instrument control
ECD	-	Electron capture detector
EPA	-	Environmental protection agency (USA)
GC	-	Gas chromatograph / gas chromatography
HS	-	Headspace analysis
HS-SPME	-	Headspace solid-phase microextraction
I.D.	-	Inner diameter
ITSP	-	Instrument-top sample preparation
ITEX	-	In-tube extraction
LC	-	Liquid chromatography
LLE	-	Liquid-liquid extraction
LLME	-	Liquid-liquid microextraction
LOD	-	Limit of detection
MDL	-	Method detection limit (according to EPA)

MEPS	-	Microextraction in packed syringe
MFC	-	Mass flow controller
MHE	-	Multiple headspace extraction
MS	-	Mass spectrometry
MSPE	-	Miniaturized solid-phase extraction
MSTFA	-	*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide
m/z	-	Mass to charge ratio
O.D.	-	Outer diameter
PAH	-	Polycyclic aromatic hydrocarbons
PAL	-	Prep and load (autosampler platform)
PDMS	-	Polydimethylsiloxane
POM	-	Particulate organic matter
P&T	-	Purge & trap
QuEChERS	-	Quick easy cheap effective rugged safe
RPM	-	Rounds per minute
SBSE	-	Stir bar sorptive extraction
SHS	-	Syringe headspace analysis
SI	-	Supporting information
SIM	-	Selected ion monitoring
SPME	-	Solid-phase microextraction
SPE	-	Solid-phase extraction
SSL	-	Split/Splitless injector
TIC	-	Total ion count

TMS	-	Trimethylsilyl(-derivative)
VOC	-	Volatile organic carbon

7.2 List of figures

7.3 List of tables

7.4 List of publications

1. Andreas Kremser, Maik A. Jochmann, Torsten C. Schmidt

   PAL SPME Arrow - Evaluation of a Novel Solid-Phase Microextraction Device for Freely Dissolved PAHs in Water

   Analytical and Bioanalytical Chemistry, January 2016, Volume 408, Issue 3, pp 943-952 [7]

7.5 Oral presentations, posters and conference attendances

No.	Date	Description	Form of attendance
1	04-07.03.2013	Anakon Conference & Expo Essen	Technical support
2	05.01.2014	PhD-Student seminar Hohenroda	Chairman
3	18-23.05.2014	ISCC Riva	Poster presentation
4	10-11.09.2014	Mülheimer Wassernalytisches Seminar	Oral presentation
5	16-18.09.2014	Axel Semrau Berlin Strausberg	Oral presentation
6	08-14.03.2015	Pittcon Conference & Expo New Orleans	Oral presentation
7	01-03.09.2015	Axel Semrau Sprockhövel	Oral presentation
8	08-10.11.2015	Langenauer Wasserforum	Oral presentation and poster presentation

7.6 Curriculum vitae

The curriculum vitae is not contained in this online version for reasons of privacy protection.

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

7.7 Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel:

Advances in Automated Sample Preparation for Gas Chromatography: Solid-Phase Microextraction, Headspace-Analysis, Solid-Phase Extraction

Selbst verfasst und keine außer den angegebenen Hilfsmittel und Quellen benutzt habe, und dass die Arbeit in dieser oder ähnlicher Form noch bei keiner anderen Universität eingereicht wurde.

Essen, im Juni 2016

Andreas Kremser

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